Contact with beach sand and risk of illness

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ABSTRACT

CHRISTOPHER DAVID HEANEY: Contact with beach sand and risk of illness
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Background: Recently, numerous studies of fecal contamination of beach sand have shown that beach sand can harbor higher concentrations of fecal indicator organisms than nearby recreational waters. Although fecal pathogens have also been isolated from beach sand, the risk of illness associated with beach sand contact and fecal indicator organism concentrations in sand is unclear. *Methods:* During 2003-2005 and 2007, beach visitors at 7 U.S. beaches were enrolled in the study and asked about sand contact the day of their beach visit. Ten to 12 days later participants were telephoned to answer questions about health symptoms experienced since the visit. At 2 study beaches in 2007, beach sand was analyzed for concentrations of the fecal indicators *Enterococcus*, *Bacteroides*, *B. thetaiotaomicron*, and F^+-specific coliphage. *Results:* We completed a total of 27,365 interviews at 4 freshwater and 3 marine water beaches. Sand contact was strongly associated with age, water contact, and beach. After controlling for age, sex, water contact, race/ethnicity, and beach, digging in the sand was positively associated with gastrointestinal (GI) illness (aIPR=1.14; 95% CI 1.02–1.26) and diarrhea (aIPR=1.20; 95% CI 1.05–1.36). The point estimate was slightly stronger between being buried in the sand and GI illness (aIPR=1.22; 95% CI 1.04–1.42) and diarrhea (aIPR=1.23; 95% CI 1.01-1.51), respectively. Similar effects were observed among nonswimmers digging in sand for GI illness (aIPR = 1.26; 95% CI = 1.03-1.55) and diarrhea (aIPR = 1.26; 95% CI = 0.98-1.62). Stronger associations were

observed among those getting sand in their mouth for GI illness (aIPR=1.82; 95% CI 1.19-2.78) and diarrhea (aIPR=1.65; 95% CI = 0.96-2.84). Non-enteric illnesses were not consistently associated with sand contact. Variation was observed in beach specific results suggesting site-specific factors may be important in the risk of illness following sand exposure. At 2 marine beaches 144 sand samples were analyzed for fecal indicators and 4,999 interviews were completed. A molecular measure of *Enterococcus* in sand (qPCR CCE/g) was positively associated with GI illness among those digging in sand (aOR per log increase in qPCR CCE/g=1.45; 95% CI 1.05-2.01) and buried in the sand (aOR = 3.12; 95% CI 1.08-9.05). The relationship between other sand fecal indicator measures with GI illness was not consistent. *Conclusions:* Contact with beach sand was positively associated with enteric illness at beach sites but there was variability in the effect by beach. This study demonstrated a positive relationship between sand contact activities and GI illness as a function of microbial sand quality.

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TABLE OF CONTENTS

LIST OF TABLES

METHODS CHAPTER IV.

RESULTS CHAPTER V. A.

RESULTS CHAPTER V. B.

LIST OF FIGURES

I. INTRODUCTION

In the United States, increasing numbers of people are moving towards coastal areas. In 2003, the National Oceanic and Atmospheric Association (NOAA) estimated that approximately 53 percent of the nation's population (153 million people) lived in the Nation's 673 coastal counties.[1] This is an increase of 33 million people since 1980.[1] In addition to increasing numbers of people moving to coastal areas, seasonal visitation of coastal beaches is a favored pastime in the United States. In a survey of >75,000 households, 40% of respondents ≥16 years of age, equivalent to 82 million individuals (extrapolated from the survey), reported visiting beaches for outdoor recreational activities during 1999-2000.[2,3] Beaches, particularly the sand and water, may become contaminated by fecal pollution from human sewage as a result of municipal waste water treatment plant (WWTP) discharges and combined sewer overflows, and diffuse sources such as urban run-off, domestic and wild animals, and human bathers.[2] It is therefore important to study the impact of fecal contamination of the beach environment on human health as a result of recreational beach activities.

In response to concerns about fecal contamination at beaches, the 106th Congress of the United States of America signed the Beaches Environmental Assessment and Coastal Health Act (BEACH Act) into law on October 10, 2000.[4] The BEACH Act amends the Federal Water Pollution Control Act with the goal of reducing the risk of disease to users of the Nation's recreational waters. The BEACH Act authorizes the

United States Environmental Protection Agency (EPA) to publish performance criteria for monitoring and assessment of coastal recreational waters and provide prompt notification of exceeding applicable water quality standards through: 1) strengthening of beach standards and testing, 2) provision of faster laboratory test methods, 3) prediction of pollution, and 4) investments in health and methods research.[4] The BEACH Act recommends that individual states with coastal areas adopt coastal recreation water quality criteria for pathogen indicator organisms based on densities of *E. coli* and/or *Enterococcus* in water. EPA works with states, tribes, and local governments to strengthen local beach health monitoring efforts and procedures to achieve these standards by providing technical guidance and training on new test methods and predictive models.

As a result of the BEACH Act of 2000, much research has focused on the risk of illness resulting from swimming exposure to pathogen indicators in recreational waters.[5-10] In 2003 the EPA and the Centers for Disease Control and Prevention (CDC) initiated the National Epidemiologic and Environmental Assessment of Recreational (NEEAR) water study, a multi-year large prospective cohort study of the association between recreational water contact (e.g., swimming) and illness among beach-goers. The results of this and other related studies have shown an increased risk of illness (including enteric and non-enteric illness) among swimmers exposed to increased levels of indicators of fecal contamination in recreational water.[5-7,10-33]

The BEACH Act does not make recommendations for monitoring and testing of sand; nor does it set guideline or criteria values for indicators of sand quality. Little is known about the relationship between human-derived fecal pollution in beach sand and

the risk of illness among beach-goers who come into contact with beach sand during recreational activities. This research summarizes the results of a two-phase investigation of the relationship between human contact with beach sand and the risk of illness at beaches influenced by a point source municipal sewage discharge. During the first phase of this research, we examined the relationship between self-reports of specific beach sand contact activities (digging in the sand or building sand castles; having one's body buried in the sand) and the risk of illness among beachgoers using data from participants in the 2003-2005 and 2007 NEEAR water studies. During the second phase, we evaluated the relationship between levels of fecal indicators of microbial pathogens (*Enterococcus, Bacteroides*, *B. thetaiotaomicron*, and F^+-specific coliphage) in beach sand and risk of health symptoms and illness reported by beach-goers participating in the 2007 NEEAR Water Study.

II. REVIEW OF THE LITERATURE

A. Critical review of literature

1. The fecal indicator concept

Fecal indicator organisms include a group of bacteria and viruses, such as total and fecal coliforms, *E. coli*, *Enterococcus*, *Bacteroides*, *Clostridium perfringens*, and bacteriophage (coliphage), whose presence in the environment indicates the presence of fecal contamination and the pathogenic organisms associated with such contamination.[34-37] Monitoring for specific pathogens is not usually considered a practical or cost-effective strategy to protect the public's health from fecal contamination in the environment (*e.g.*, water, sand, sediment, air).[34-37] There are too many pathogens to incorporate into routine monitoring and surveillance. Individual pathogens are usually present in low concentrations and often require analysis of large sample volumes. Methods for detecting pathogens are also generally expensive, technically demanding, and time-consuming.[36] Microbiologists routinely examine organisms whose presence in the environment (e.g., water, sand, sediment, air) indicates the presence of fecal contamination and pathogens associated with such contamination. These organisms are classified as fecal indicator or index organisms. The indicator concept involves quantifying microbes (or chemical compounds) that are non-pathogenic and commonly occur in the feces of humans and warm-blooded animals. The presence of these non-

pathogenic indicator organisms suggest that fecal contamination has occurred and that harmful pathogenic organisms may also be present. Methods for the quantification of fecal indicator organisms in the environment are generally less expensive, faster, and easier to perform in most laboratories.

Bonde (1966), Goyal, (1983), and Stetler (1984) have contributed greatly to the body of research on fecal indicator organisms. According to their observations, the ideal fecal indicator should:

1) be present in feces, sewage, and fecally contaminated samples when pathogens are present;
2) be absent in non-fecally contaminated samples and when pathogens are absent;
3) occur in much greater numbers than pathogens;
4) occur in a constant ratio to pathogens (numbers should correlate with amount of fecal pollution);
5) be incapable of "regrowth" or "aftergrowth" in the environment;
6) have survival/persistence greater than or at least equal to pathogens in natural environments and treatment processes;
7) be easily detected/quantified by simple laboratory tests in a short time;
8) have constant characteristics;
9) be harmless to humans and other animals;
10) have numbers in water and other media that are associated with risks of enteric illness (dose-response relationship); and
11) be applicable to all types of water (and other environmental samples). [34-37]

No single fecal indicator organism meets all of the criteria and often a grouping or suite of these indicators can be very useful to quantify levels of fecal contamination and pathogens in the environment (e.g., water, sand, sediment, air). Many fecal indicator organisms, including several groups of indicator bacteria and viruses, can provide a fitting estimation of health risks for the general population (e.g., healthy adults) and also for sensitive sub-populations (e.g., infants, children, the elderly, and immunocompromised individuals).

2. Health effects water quality indicators

The fecal indicator concept grew out of a need to effectively monitor the quality of surface and drinking water supplies with the goal of improving decision-making to prevent waterborne disease. Each fecal indicator organism and its accompanying measurement method(s) has advantages and limitations. The literature suggests that there is no single gold standard water quality fecal indicator to predict health symptoms and illness.[38] In *Water Pollution Microbiology*, Volume 2 (1978), Cabelli defines a health effects water quality indicator as "some microbial, chemical, or physical parameter which indexes the potential risk of infectious disease coincident with people's use of the aquatic environment as a source of water, recreation, or food. In the final analysis, the best indicator—there is no ideal—is the one whose densities correlate best with health hazards associated with a given (preferably several) type of pollution".[39] Cabelli suggests that health effects water quality indicators can be screened against several criteria.[39] These criteria parallel the criteria for fecal indicator organisms identified by Bonde (1966), Goyal, (1983), and Stetler (1984) but are tailored to indicators that are best for prediction of health effects. Cabelli suggests that, "the indicator: (i) should be consistently and

exclusively associated with the source of pathogens; (ii) must be present in sufficient numbers to provide an "accurate" density estimate whenever the level of each of the pathogens is such that the risk of illness is unacceptable; (iii) should approach the resistance to disinfectants and environmental stress, including deposited toxic materials, of the most resistant pathogen potentially present at significant levels in the source; and (iv) should be quantifiable in recreational waters by reasonably facile and inexpensive methods and with considerable accuracy, precision, and specificity".[39]

Cabelli's criteria for health effects water quality indicators has relevance for the future development of a health effects beach sand quality indicator. Currently little epidemiologic information exists characterizing associations between concentrations of fecal indicators in beach sand and health symptoms and illness. The literature suggests that several (i.e., a group or suite) of the fecal indicators may be useful as predictors of health outcomes experienced by beach-goers who come into contact with beach sand.[38,40-45] Numerous recent studies suggest that *Enterococcus*, *Bacteroides*, and F^+-specific coliphage could be candidate health effects sand quality indicators. To provide background for the use of *Enterococcus*, *Bacteroides*, and F^+-specific coliphage as health effects beach sand quality indicators during these research activities, a review of several classes of fecal indicator organisms and their utility as a model of the presence of human-derived fecal pathogens in various media (e.g., water, sand, sediment) is presented.

a. Total coliforms and fecal coliforms

Methods for detection of coliform bacteria, which include total coliforms, thermotolerant or fecal coliforms, and *E. coli*, are codified throughout the world and commonly accepted to quantify fecal contamination in drinking, waste, marine, and fresh

water, and other media (e.g., soil, sediment, sand, air) samples.[34-36,46,47] Coliform bacteria include *Escherichia*, *Enterobacter*, *Citrobacter*, and *Klebsiella* species. Coliform bacteria are traditionally defined as aerobic and facultatively anaerobic, gram-negative, non-spore forming, rod-shaped bacteria that ferment lactose with gas and acid production in 24 to 48 h at 35ºC.[36,47-52] Total coliform bacteria possess the enzyme *β*-galactosidase. Total coliforms can originate from non-fecal environmental sources such as plants and soils whereas thermotolerant or fecal coliforms and *E. coli* mainly have been shown to restrict enumeration to coliforms of fecal origin.[48,53,54]

Fecal or thermotolerant coliforms, a more definitive indicator of homeothermic fecal contamination, grow and ferment lactose with the production of gas and acid at 44.5 ± 0.2ºC.[47] Studies have shown that coliform bacteria are found in contaminated water at densities roughly proportional to waterborne fecal pollution.[48] Coliform bacteria also have demonstrated longer or similar survival and viability in the environment than disease-causing or pathogenic bacteria. Historically, for this reason it has been thought that the absence of coliform bacteria in water suggests that it is safe for human use.[48] However, a recent review of the regulatory reliance upon total coliform monitoring (as required by the Total Coliform Rule of 1990) is raising questions regarding its use to prevent waterborne disease outbreaks.[49,54,55] There have been major waterborne disease outbreaks where coliform bacteria have been absent or present at allowable concentrations.[50,51] The most severe example is the 1993 epidemic of cryptosporidiosis in Milwaukee, Wisconsin, caused by the protozoan parasite *Cryptosporidium*, in which more than 400,000 individuals became ill.[56] The survival (of disinfection processes) and persistence (in the environment) of coliform bacteria do not correlate with the survival of

certain pathogens, including enteric viruses belonging to the *Picornoviridae*, *Adenoviridae*, and *Caliciviridae* families and protozoan (oo)cysts, such as *Cryptosporidium parvum* and *Giardia lamblia*. For this reason, coliform bacteria may be more appropriate indicators of enteric bacterial pathogens such as *Campylobacter*, *Salmonella*, *Shigella*, *Yersinia*, and *Vibrio*.

b. *Escherichia coli*

A common problem with the use of total and fecal coliforms, which include the genera *Escherichia* and *Klebsiella*, is that their presence does not differentiate between animal, human, and other sources of fecal contamination. Fecal coliforms, specifically the *Klebsiella* genus, can also originate from non-fecal environmental sources such as carbohydrate-rich industrial effluent and vegetative material.[57] Although *E. coli* is a member of the fecal coliform group of bacteria, it has been demonstrated to be a more specific indicator for the presence of homeothermic fecal contamination.[58] It can be enzymatically distinguished by the lack of urease and the presence of β-glucuronidase.[47] The EPA has adopted recreational freshwater quality criteria for bacteria based upon *E. coli* as an indicator of fecal contamination.[59] Epidemiologic and microbiologic studies summarized by EPA in the *Health Effects Criteria for Fresh Recreational Waters* demonstrated a positive linear relationship between swimming-associated gastroenteritis and *E. coli* concentrations.[60] However, *E. coli* has been not been shown to be effective at predicting gastrointestinal (GI) illness in marine waters because it dies off quickly, making it an unsuitable fecal indicator in the marine environment.[61-67] Additionally, in tropical environments, *E. coli* has been detected in pristine forest aquatic and plant systems and several studies suggest that *E. coli* may be able to replicate in the sand,

sediment, and soils of temperate regions.[68-72] Therefore its use in tropical and equatorial regions of the world (and during the hot summer months of more temperate regions) may not be appropriate as an indicator of fecal pollution.[47,51]

c. *Enterococcus* and *Bacteroides*

Given some of the limitations of the coliform group (e.g., total and fecal coliforms and *E. coli*) as indicator organisms, other *Enterobacteriaceae* have been proposed as indicators. These include enterococci such as *Enterococcus faecalis* and *E. faecium* and other anaerobes such as *Bacteroides* spp. and *Bifidobacteria* spp. *E. faecalis*, and *E. faecium* are gram-positive bacteria commonly inhabiting the intestinal tracts of humans and animals.[73] *Bacteroides* are a genus of gram-negative, rod-shaped bacteria. *Bacteroides* are obligate anaerobes and as many as 10^{10}-10^{11} cells per gram of human feces have been reported.[74] *Bacteroides* spp. dominate the human intestinal flora, and because research has shown that some species (*B. thetaiotaomicron*) mainly only live in the human intestine, these bacteria may be useful to distinguish human from nonhuman sources of fecal contamination.[67,75-77] *Enterococcus* and *Bacteroides* are more resistant than coliforms (including *E. coli*) to chlorine disinfection during the sewage treatment process and *Enterococcus* survives for longer periods in the marine environment, making them attractive for use as indicators of groups of fecal pathogens that survive sewage treatment.

The enterococci group is a subgroup of the fecal streptococci that are differentiated from other streptococci by their ability to grow in 6.5% sodium chloride, at pH 9.6 and at 10°C and 45°C.[47,78] The term fecal *Streptococcus* is synonymous with Lancefields's group D *Streptococcus,* which include *E. faecalis, E. faecium, S. bovis,* and

S. equinus. *E. faecalis* and *E. faecium* are the most commonly detected *Enterococcus* inhabiting the human intestinal tract and thought to be more human specific indicators of fecal contamination, especially in marine waters.[59] Consequently the EPA has adopted recreational marine water quality criteria for bacteria based upon *Enterococcus* as an indicator of fecal contamination.[59] EPA's recreational marine water quality guideline for *Enterococcus* is based on bacterial density and is 35 *Enterococcus* /100 ml.[51] Geldreich (1978) found that *fecal streptococcus* counts greater than 100 /100 ml indicate significant fecal pollution derived from a warm-blooded animal source.[11,15,27] Several studies have shown that *Enterococcus* are more accurate indicators of swimming-associated health risks than members of the coliform group.[11] In 1982 Cabelli et al., found that among swimmers vs. non-swimmers at beaches with varying water quality in New York City, Boston, and Lake Pontchartrain, LA, the numbers of *Enterococcus* most closely correlated with the appearance of gastrointestinal symptoms following exposure to fecally polluted waters.[43,79-81]

Recently studies have suggested that sand and sediment may serve as a reservoir for *Enterococcus*, as greater numbers of *Enterococcus* have been found in sand compared to bathing waters.[10,67] Findings of the retention and possible replication of *Enterococcus* in beach sand, particularly in tropical climates, raise questions about the appropriateness of its use and the specificity of this indicator for determining recent sewage contamination. Because of these findings Fujioka (2001 & 2006) recommends the use of alternate bacterial indicator organisms, such as *Clostridium perfringens*, and viral indicator organisms, such as FRNA and FDNA coliphages (non-pathogenic viruses that infect and replicate in *E. coli* bacteria), because non-point source fecal contamination

(i.e., of non-sewage origin) may dominate in tropical waters, sands, and soils.[61,67] Data and results from exposure assessment and epidemiologic studies involving these novel indicators have not been sufficiently compelling for EPA to change its national recreational water quality criteria (which are based on densities of *E. coli* and *Enterococcus*).[82] However, F^+-specific coliphage will be discussed in the *Bacteriophage* section below.

In temperate marine waters and beach environments where sewage point sources dominate, *Enterococcus* can serve as an effective indicator of fecal contamination. Additionally, given supportive epidemiologic evidence (e.g., dose-response relationship), approval by EPA for its widespread use, and standardized detection methods, *Enterococcus* is an attractive health effects sand quality indicator for the proposed study of the association between sand exposure and health effects at marine beaches.

d. *Bacteriophage*

Much research has focused on the use of bacteriophage as an alternate indicator to traditional fecal bacterial indicators such as coliforms, *E. coli*, and *Enterococcus*. The use of bacteriophage as a health effects water quality indicator arose partially due to studies documenting their greater survival and resistance to disinfection during the sewage treatment process than bacterial indicators (e.g., total and fecal coliforms, *E. coli*, *Enterococcus*, *Bacteroides*)[83] and enteric viruses such as poliovirus[84,85]. Bacteriophage are present in higher concentrations than enteric viruses in fecally polluted environmental waters and are considered useful as indicators of pathogenic enteric viruses and sewage contamination. Bacteriophage are ubiquitous in human and animal feces and in sewage contaminated waters.[86,87] Significant correlations (0.999) between bacteriophage and

coliform bacteria in freshwater show that bacteriophage can be used to indicate the sanitary quality of water.[88]

Bacteriophage are viruses that infect, replicate in, and subsequently lyse bacterial host cells such as *E. coli*.[88] They consist of a nucleic acid molecule (RNA or DNA) surrounded by a protein coat or capsid.[88] FRNA bacteriophage have received the most attention and study because they are usually present in higher concentrations than FDNA bacteriophage and have morphological characteristics most similar to enteric viruses. Bacteriophage that infect *E. coli* are called coliphage. The two main coliphages of interest as indicators of sewage contamination and pathogenic enteric viruses are somatic and male-specific coliphages. The main difference between these two groups of coliphages is their mechanism for attachment to *E. coli* host bacterial cells. Somatic coliphages infect *E. coli* through direct attachment to receptor sites on the bacterial cell membrane or cell wall.[88] Male-specific coliphages infect *E. coli* host cells through receptors on the *E. coli* F pili (i.e., F^+-specific coliphage). Somatic coliphage have been detected in the feces of humans, cattle, pigs, chickens, and other animals. Male-specific or F^+-specific coliphage have been detected in feces from cows, pigs, and humans. A beneficial aspect of using bacteriophage as fecal indicators is the ability to detect and enumerate low numbers of bacteriophage in environmental water samples through enrichment methods. The EPA has accepted Method 1601, a two-step enrichment method for the detection of F^+-specific coliphages in water and other media, as a standard method.[89] Enrichment involves adding host bacteria (somatic or male-specific) and nutrients to a sample, and then incubating the sample for 16- to 24-h under conditions that permit infection of the bacteria and multiplication of the indigenous phages.[90]

Selection of the proper host bacteria is of critical importance to achieve consistent results using the enrichment method.

Wild *E. coli* strains are not considered consistent and reliable host cells for study of coliphages. A commonly studied male-specific or F^+-specific host strain is F-Amp.[91] The F-amp host is resistant to both ampicillin and streptomycin, a characteristic that minimizes overgrowth by indigenous bacteria in environmental samples.[92] The F-amp host is more attractive for environmental samples relative to somatic hosts such as C3000 because it reduces the interfering indigenous background bacteria present in water, sand, and sediment. F-amp host has added utility because genotypic and phenotypic methods can be performed on coliphage that infect it. This allows for source tracking of observed fecal contamination (e.g., differentiation between animal vs. human-specific sources). Molecular techniques were developed by Furuse et al (1981) to serotype F^+-specific RNA coliphages into four groups (I-IV) revealing that serogroup II and III phages tend to be isolates of human feces, whereas group I are usually of animal origin and group IV is of mixed origin.[92] Havelaar et al, (1990), also found that group II and III phages were present in high concentrations in human sewage.[92] In human sewage, both somatic and male-specific coliphages have been found to be present at concentrations of around 1000 PFU/100ml.[10,18,43-45,61,63,64,66,68-70,79,93-112] However, concentrations of somatic coliphages in sewage can range up to 15,900 PFU/ml.[10,12,18,43,44,61,93,98,101,102,104,113-117] In a review of coliphages as indicators of pathogenic enteric viruses, Leclerc et al (2000) found, through observations on septic tanks, that F^+-specific coliphage concentrations were not high.[118] In their review they also found that only 3% of humans have *E. coli* with F^+-specific or FRNA coliphages.[43] Still, the advantage of typing FRNA coliphages is considerable.

The high specificity of FRNA coliphage (e.g., groups II and III) to human sources of fecal contamination outweighs the disadvantage of possible non-detection due to low numbers in the environment. Somatic coliphages are not attractive as indicators because studies have shown that they may naturally occur in the environment. There is also a lack of correlation between somatic coliphages and enteric viruses.[43,94,99,103,119-126] Use of somatic coliphage as a health effects sand quality indicator is problematic because of its lack of specificity to sewage discharges.[118] However; compared to the bacterial indicators presently recommended by EPA (e.g., *E. coli* and *Enterococcus*), both somatic and F^+-specific coliphages are better models of pathogenic enteric viruses in the marine environment.[44,61,65,68-70,79,93,95,96,98,104,109-111,123,127-130]

3. Applications to health effects sand quality indicators

The above discussion of health effects water quality indicators provides the background for selection of fecal indicator organisms that are appropriate health effects sand quality indicators. The literature shows that each fecal indicator has a variable sensitivity and specificity to mark the presence of fecal contamination from a known sewage discharge into the marine environment. Additionally, the literature shows that each fecal indicator has a variable sensitivity and specificity to groups of pathogenic organisms (viruses, bacteria, and protozoan parasites) that are known to have variable resistance to disinfection during the sewage treatment process.

No single indicator organism can be considered a gold standard. Total and fecal coliforms, *E. coli*, and somatic coliphage have poor specificity to human sewage in the marine environment. Total and fecal coliforms, *E. coli*, and somatic coliphage therefore are not attractive candidate health effects sand quality indicators. Of the group of

bacterial indicators discussed above, *Enterococcus* and *Bacteroides* are more resistant to chlorine disinfection during sewage treatment. All of the bacterial indicators, however, are more susceptible than F^+-specific coliphage to inactivation by chlorine disinfection during sewage treatment.[131-135]

Bacterial indicators may not be adequately sensitive to mark the presence of pathogenic enteric viruses and protozoa because bacterial cells die-off more readily during sewage treatment.[131-135] Traditional bacterial culture methods measure live bacterial cells in the marine environment (e.g., water and sand). A novel method of detection of *Enterococcus* and *Bacteroides*, quantitative TaqMan polymerase chain reaction cell equivalents (QPCR CCE), measures both live and dead bacterial cells in water and sand.[136] This detection method is a more sensitive indicator of the presence of viral and protozoan pathogens that survive sewage treatment and improves upon the limitations of culture-based bacterial assays.[9]

Considering the advantages and limitations of each indicator, it is most appropriate to use a suite of fecal indicators that include the following bacteria (e.g., *Enterococcus*, *Bacteroides*) and viruses (F^+-specific coliphage).[38] Additionally, considering the limitations of the bacterial culture-based methods it is appropriate to use a novel QPCR CCE method for measurement of *Enterococcus* and *Bacteroides*. This approach will maximize the advantageous properties of the indicators and provide a good balance between sensitivity to detect fecal contamination when it is present in the marine environment and specificity to identify the source of fecal contamination as a known municipal sewage outfall.

4. Abundance of fecal indicator organisms and pathogens in beach sand

Recently, numerous exposure assessment studies have triggered interest amongst scientists, the news media, and the general public concerning levels of fecal contamination in beach sand.[22,40-45,65,68,69,99,110,120,122,123,137,138] These studies consistently showed high concentrations of fecal indicator organisms in beach sand and sediment during the summer swimming season and also throughout the year. Some studies attributed the source of the fecal indicator organisms to known municipal sewage discharges in close proximity to the beaches; however, other studies attributed the source of fecal contamination to warm-blooded domestic and wild animals.[43,68,110,111] Shiaris et al., found that fecal coliforms were present in sediments at abundances 2 to 4 orders of magnitude higher than in the overlying water column.[109] Wheeler Alm et al., observed a mean summer abundance of *E. coli* and *Enterococcus* 3-38 times as high in the top 20 cm of wet-sand cores compared to levels in the water column at six freshwater bathing beaches on Lake Huron, MI.[68,110,111]

In addition to fecal indicator organisms, several studies have detected pathogenic bacteria (*Pseudomonas aeruginosa*, *Salmonella*, *Shigella*, *Campylobacter jejuni*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, and *Vibrio harvey*), viruses (adenovirus, norovirus, enterovirus, hepatitis A virus), fungi (*Candida albicans* and dermatophytic fungi), protozoan parasites (*Cryptosporidium parvum*, *Giardia lamblia*), and parasitic nematodes (*Toxocara canis*) in beach sand.[64,65,68-70,96,98,128] Numerous studies found that the conditions in foreshore, nearshore, and backshore sand favor the persistence, survival, and possible re-growth of *E. coli* and *Enterococcus* suggesting that elevated levels of these fecal indicator bacteria (FIB) in sand may represent

autochthonous populations rather than impacts from sewage sources of contamination.[32,41,124] The conditions that favor persistence, survival, and possible re-growth of FIB in sand include increased protection from sunlight, buffered temperatures, more nutrient availability, reduced osmotic stress, cover from predation by other microorganisms, a large surface area for biofilm development, and higher moisture and organic content from wave swash.[32,45] Some studies suggest that conditions in nearshore wet sand may be more favorable for FIB survival than backshore dry sand[32,33]; however, others suggest that dry sand conditions favor the ability of *E. coli* to outcompete predators and survive as an autochthonous population.[124] The literature suggests that foreshore wet sand is more directly impacted by municipal sewage discharges whereas backshore sand is more impacted by fecal contamination from wild animals, domestic pets, and human activities (such as bathhouses, showers, and restrooms).[41,43,94] At any specific beach, it is not entirely clear which bacterial sources initially populate the sand bacteria community, but it is clear that bacterial and viral indicators of fecal pathogens (including *E. coli*, *Enterococcus*, *Bacteroides*, and F^+-specific and somatic coliphage) are present orders of magnitude higher in wet beach sand compared to nearby bathing waters; and that sewage discharges directly impact wet nearshore sand via wave swash.[40,41,43]

5. Beach sand and illness

Recent exposure assessment studies have shown that fecal indicators, specifically *E. coli* and *Enterococcus*, and fecal pathogens are present at high concentrations in beach sand. However, neither EPA nor States currently recommend any monitoring criteria for sand quality. This raises important questions regarding the safety of human contact with beach sand and whether sand exposure poses an increased risk of illness for beach-goers.

Although numerous exposure studies have provided useful information for water and sand quality assessments, the relationship between contact with sand among beach-goers and illness has not been properly evaluated through a full-scale epidemiologic study.[6,7,9,15,16,21,22,139]

In 1985, Seyfried et al. collected water and sediment samples up to three times a day at ten beaches in Ontario, Canada and analyzed them for fecal coliforms, fecal streptococci, coagulase-positive and coagulase-negative staphylococci, *Pseudomonas aeruginosa*, and heterotrophic bacteria.[140] Their primary research question focused on comparing the incidence of illness among those exposed and unexposed to fecal indicators in water (*i.e.*, comparing swimmers to non-swimmers). The authors used linear regression models to explore associations. However, during the course of this study it was observed that FIB densities were found to be approximately 10 times higher in the sediment than in the corresponding surface water samples.[18] To investigate this secondary finding, the authors used linear regression models to explore associations between FIB concentrations in sand and self-reported gastrointestinal (GI) illness. The authors reported no association between levels of FIB in beach sediment samples and GI illness. From their description of the sand exposure assessment, it appears that Seyfreid et al. did not perform a detailed sand exposure assessment. The study lacked detailed questions of participants and their activities involving contact with sand, information on sand sample distribution, sample collection method, and number of samples. Seyfried et al. interviewed a total of 8,402 people, of which only 3,967 swimmers and 2,105 non-swimmers provided complete information. The authors had missing information for 28% of their sample. If the individuals with missing information were systematically different

from those with complete information then their findings could have been biased. The authors also do not report the percentage of participants exposed and unexposed to contact with beach sand and details of their method of classifying exposure to sand. Their study ancillary investigation of sand exposure does not provide adequate answers to the research question regarding sand exposure and its association with illness.

Similar to Seyfried et al., Marino et al. (1995) collected water and sand samples at a beach in Malaga, Spain and interviewed beach goers about illnesses they experienced after recreational beach activities.[9] Water samples were tested for total coliforms, fecal coliforms, *Escherichia coli*, fecal streptococci, *Clostridium perfringens*, coliphage, *Salmonella* spp., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Vibrio* spp., and *Candida albicans*. As a secondary research endeavor, sand samples were collected and tested for fecal indicators, *Candida albicans*, and dermatophytic fungi only. The investigators examined the potential association between fecal indicator levels in sand and illness. Their results did not demonstrate an association between self-reported contact with beach sand and illness or between increasing levels of fecal indicators in sand and illness. The investigators do not report details of their sand exposure assessment methods (e.g., sand sampling distribution, frequency, collection method). It appears that the study lacked detailed questions on participants' activities involving contact with sand, information on sand sample distribution, sample collection method, and number of samples. The authors state that they, "selected a representative sample of the total population (9,691 persons, of them 6,157 were locals and 3,534 tourists) that was not affected by confounding factors, such as: bathing in another beach, bathing in swimming-pools, presence of any allergic processes, problems related to toxi-

infections by contaminated food and/or beverages, etc".[124] They present the sample population in Table 1 of the manuscript. Although 9,691 individuals were said to be selected from the source population, totals from Table 1, indicate that only 2,463 exposed (swimmers) and 304 unexposed (non-swimmers) were included (a total of 2,767 individuals) in their analysis of swimming-associated illness. The authors limited their sample size and their power to detect effect by initially selecting a representative sample and then further restricting their study population. Marino et al. (1995) report that they did not observe an effect between sand exposure and illness, but the authors do not report the number of individuals exposed and unexposed to beach sand. It is likely that their sub-group analysis of sand exposure and illness suffers to a greater extent from small numbers of individuals in categories of exposed and unexposed to sand. Marino et al.'s (1995) finding of a lack of an association between contact with beach sand and illness must be interpreted cautiously due to likely poor statistical power to detect an effect.

Recently Bonilla et al., (2007) conducted an exposure assessment and pilot epidemiological study of the prevalence and distribution of fecal indicator organisms in water and sand at three South Florida marine beaches.[141] The authors observed that "indicator organisms were statistically elevated in sand relative to water," and decided to consider "the potential health risks associated with beach use and exposure to sand".[44,61,113,125] Over a two-year period, Bonnilla et al., measured fecal coliforms, *E. coli*, enterococci, somatic coliphages, and F^{+}-specific coliphages in water and sand at Ft. Lauderdale Beach, Hollywood Beach, and Hobie Beach in South Florida. The authors also conducted two experiments: one to assess the impact of gull excrement on sand and another to model movement of FIB by people walking across dry sand on the beach. For

the latter the authors tracked the spread of fluorescently dyed beads the size of fecal indicator bacteria to assess the spread of fecal indicator organisms across sand. They also examined the microspatial distribution of *Enterococcus* in both wet and dry beach sand at short intervals along 2m transects. This study involved a more detailed exposure assessment by measuring fecal indicator levels in sand and investigating the fate and transport of fecal indicators in sand. The findings of the exposure assessment experiments and activities support the findings of previous studies. FIB were found at higher concentrations in sand relative to water. FIB were also found at higher concentrations in dry sand relative to wet sand, but, their results suggest that the high levels in the dry sand are due to animal (e.g., gull) fecal inputs. Bonilla et al. described their pilot prospective cohort study with insufficient detail and did not present details of their study design including the selection of the study population, exposure and outcome classification, and data analysis methods. The authors stated that the study population consisted of 882 individuals in an experimental group and 609 individuals in a control group. The authors also report that the control group consisted of non-beach-goers randomly chosen from the general population who had not visited a beach in at last 9 days. They reported that crude rates of gastrointestinal (GI) illness were higher among controls (15.3/100) relative to beachgoers (8.5/100). Because rates of GI illness were higher among the control group, the investigators excluded the controls and calculated odds ratios based upon the number of minutes beach-goers' spent in the wet sand (1.008 (95% CI 1.001-1.015) and the number of minutes spent in the water (1.009 (95% CI 1.000-1.018)). The effect estimates were provided without supporting tabular data illustrating the number of individuals who refused participation, the percent of

respondents excluded due to missing data, and the number of participants included in the analyses by exposure, outcome, and covariate(s) status. The authors report a positive dose-response relationship between the number of minutes spent in the water and the number of minutes spent in the wet sand and GI illness, respectively. The limitations of Bonilla et al.'s (2007) pilot epidemiologic study include: 1) a small study population (i.e., likely poor statistical power to examine subgroups or effect measure modification); 2) selection of and exclusion of external control group that was not comparable to beach-goers; 3) a lack of details describing their study design features (i.e., exposure assignment, exposure and outcome classification); and 4) lack of details describing their epidemiologic data analysis methods (e.g., exposure assignment, exclusion of controls from the analysis; lack of adjustment for covariates of interest). These limitations diminish the study's contribution to addressing the question of whether contact with beach sand is associated with an increased risk of illness.

The studies by Seyfried et al. and Marino et al. were designed with a primary focus on water quality and swimming-associated illness; not on sand quality and sand-associated illness. Seyfried et al. and Marino et al. also did not perform a detailed sand exposure assessment. They did not include detailed questions about the extent of sand exposure including activities such as playing in the sand, digging in the sand, having one's body buried in the sand, hand-washing after playing in the sand, and eating with one's hands after playing in the sand. Such lack of detail in the planning of sand exposure assessment can result in exposure misclassification and in certain cases attenuate effect estimates towards the null (e.g., non-differential exposure misclassification).[142] Although Bonilla et al., performed a more detailed exposure

assessment with respect to fecal indicator sand sampling, the epidemiologic aspects of the study were lacking as evidenced by their selection of an external control group, poor statistical power to detect an effect (i.e., small sample size), and lack of adjustment for potential confounders during data analysis.

The epidemiologic studies reviewed above leave unresolved questions concerning sand exposure and its association with illness. The questions that are unresolved could be addressed by a study that included: 1) a detailed exposure assessment that includes a sand activities questionnaire and fecal indicator sand sampling; 2) a sufficiently large sample size to give reasonable statistical power to detect an effect; 3) appropriate epidemiologic study design features to reduce the potential effect of systematic errors (e.g., measurement of exposure and confounders of interest before outcome measurement, measurement of outcomes of interest at baseline and after a follow-up period, selection/use of an appropriate control group); and 4) appropriate data analysis methods to control for covariates that are known confounding factors or effect measure modifiers. A well-powered epidemiologic study with appropriate design features and data analysis methods is needed to address unresolved questions raised by the literature on the relationship between beach sand exposure and illness at beaches with a known point source of sewage contamination.

The health effects [59], economic burden [59], and severity of illness [93,143-145] associated with bathing in fresh and marine recreational waters contaminated with domestic sewage have been well-studied. In 2006, Wade et al. showed that rapidly measured *Enterococcus* in recreational waters is predictive of swimming-associated GI illness.[9] Wade et al. (2006) reported results from the 2003-2004 NEEAR water studies at

the Great Lakes freshwater beaches. Their analyses focused first on the association between swimming contact and GI illness. The authors observed that those with any water contact were almost twice as likely to have GI illness compared with nonswimmers [adjusted odds ratio (AOR) = 1.96; 95% confidence interval (CI), 1.33-2.90]. [9] Next, the authors evaluated the association between *Enterococcus* levels in water, measured by quantitative TaqMan polymerase chain reaction cell equivalents (qPCR CCE) per 100 ml, and GI illness.[9] Wade et al. (2006) used daily averages of *Enterococcus* qPCR CCE to characterize the association with gastrointestinal (GI) illness. Wade et al., (2006) reported a positive exposure-illness relationship between rapid *Enterococcus* qPCR CCE counts and GI illness.[9] A $\log_{10}$ increase in *Enterococcus* qPCR CCE was associated with a 1.37 (95% CI, 1.10-1.71) increase in the odds of GI illness.[9] Wade et al. (2006) examined the association between exposure to a single fecal indicator (i.e. qPCR CCE counts of *Enterococcus*) and risk GI illness among swimmers and non-swimmers.[9]

Wade et al., (2008) reported that children at 10 years or younger were at greater risk for GI illness following exposure to swimming.[146] Research has demonstrated a positive relationship between increased levels of FIB at marine beaches and swimming-related illness. The relationship between contact with beach sand and health outcomes remains unresolved and has not been properly evaluated through a well-powered epidemiologic study. These research activities build and improve upon the work described in the literature and employ study design features and data analysis methods that can advance the state of knowledge concerning the safety of contact with sand during recreational beach activities.

B. Synopsis and Summary

The literature suggests that beach sand may play an important role as a contributory factor to health effects experienced by beach-goers as a result of recreational beach activities. There is mounting evidence that sand harbors high concentrations of fecal indicators and pathogens. Wheeler Alm et al. observed that the mean summer abundance of *Enterococcus* was 3-38 times as high in the top 20 cm of wet-sand cores than in the water column at six freshwater bathing beaches on Lake Huron, MI.[43] Similar findings by other investigators have prompted interest in the question of whether sand can serve as a vehicle for transmission of pathogenic microorganisms and increase the occurrence of illness following contact with sand during recreational beach activities. Past studies, however, have focused primarily on exposure assessments of sand via measurement of microbes and have not fully investigated relationships with health outcomes among individuals in contact with sand during recreational beach activities. Potential health risks have not been properly evaluated through an epidemiological study.[32,33,41,124] The following two-phase study examines physical health symptoms and illness [including gastrointestinal (GI) illness, upper respiratory illness (URI), urinary tract infection, skin rash, eye ailment, ear ache, and infected cuts] experienced by beach-goers in contact with sand and the relationship of these outcomes to health effects sand quality indicators (i.e., *Enterococcus*, *Bacteroides*, *B. thetaiotaomicron*, F^+-specific coliphage) measured in sand in which human fecal (sewage) sources dominate. These illnesses were selected because they have been shown to be associated with recreational water exposure in previous studies.[9,10]

It is clear that the sand sampling distribution (i.e., nearshore wet vs. backshore dry beach sand) is an important factor to consider so that variability of human sewage contamination can be captured and then used to assign exposure to participants of the NEEAR water study. The sand sampling approach (as well as the fecal indicator laboratory analytical methods) will be described in detail in the Methods section. Sampling wet sand in the nearshore region of the beach (e.g., 1 m from the waterline) captures the variability of fecal contamination from a municipal sewage point source.[43,68] Nearshore wet sand is impacted by wave swash in the surf zone, whereas dry sand in the backshore region of the beach is not usually affected by wave swash (except during rare hurricane storm surges). A point source of fecal contamination from a municipal sewage outfall has more impact on the wet sand in the surf zone through wave action than dry backshore sand.[68] Sampling dry sand in the backshore region of the beach captures the variability of fecal contamination from non-point animal-sources (*e.g.*, droppings of wild bird populations, domestic pets, other animals).[43] To best characterize fecal contamination from a known sewage outfall, wet nearshore sand (e.g., 1 m from the waterline) should be sampled.

The fecal indicators considered for this research include those that are regulated by EPA through current or proposed guidelines for recreational marine surface waters. *Enterococcus* was adopted by EPA in 1986 as a fecal indicator bacteria (FIB) for routine monitoring to meet marine surface water quality criteria.[11,15,39,59,60] EPA's ambient water quality guideline of 35 *Enterococcus* /100 ml for recreational marine waters is based on bacterial density.[9,136] *Enterococcus* is a fecal indicator whose presence in water suggests warm-blooded animal or human fecal contamination. The *Enterococcus* laboratory

analyses described in and the Methods section below will cover measurements used during phase two. Briefly, two methods will be used. The first is EPA Method 1600 for the enumeration of *Enterococcus*, an overnight culture-based method, approved as a standard method for the quantification of *Enterococcus* in water and other media. Method 1600 measures live culturable *Enterococcus* bacterial cells and the process requires overnight enrichment, taking up to 48 hours to get results. The *Enterococcus* qPCR CCE measurement method is included in this research because it may better reflect the presence of human pathogenic viruses and protozoa that survive wastewater treatment and also because it offers rapid results reporting (i.e., within ≤2 hours results) greatly improving public health decision-making. The rapidity of the assay is advantageous because high levels of fecal contamination detected in the morning (e.g., an 8:00 am sample) allow beach staff to make decisions about beach closures and advisories the same day of sample collection; thereby protecting beach-goers from same-day exposures to high levels of fecal contamination. Although Method 1600 and qPCR CCE both are measures of *Enterococcus*, the methods provide two different measures of the quality of the sand. Method 1600 positive results indicate the presence of viable *Enterococcus* bacteria and suggest the presence of viable bacterial pathogens. *Enterococcus* qPCR CCE positive results indicate the presence of both viable and non-viable *Enterococcus* (qPCR CCE results reflect the presence of genetic fragments of *Enterococcus*). *Enterococcus* cell fragments that are inactivated during treatment of sewage effluent are detectable by qPCR CCE analysis. Investigators at the US Environmental Protection Agency (EPA) National Exposure Research Laboratory (NERL) in Cincinnati, Ohio completed Method 1600 and molecular analyses. This analysis method has been

proposed as a better indicator of the presence of viral and protozoan pathogens in sand because these pathogens also survive treatment of sewage effluent.

F^+-specific coliphage were chosen because, relative to *Enterococcus*, they are more resistant to disinfection during sewage treatment and have been shown to be more suitable indicators of the potential presence of viral enteric pathogens.[147-149] $F^=$-specific coliphage analyses were completed at the Laboratory of Environmental Health Microbiology and Virology at the University of North Carolina at Chapel Hill. *Bacteroides thetaiotaomicron* is included because it is considered to be a more specific indicator of human sewage contamination.[150] Each of the indicators discussed (and associated detection methodologies) has limitations (such as the ability to differentiate between fecal sources) and these must be weighed against cost and ease of laboratory processing. The use of multiple fecal indicator organisms (i.e., *Enterococcus*, *Bacteroides*, *B. thetaiotaomicron*, and F^+-specific coliphage) reflects the fecal indicator criteria set forth by Cabelli (1978) which are adapted and applied to this research as a suite of health effects sand quality indicators. Previous studies' use of multiple fecal indicators to model the association between illness and fecal contamination in marine water makes this approach warranted.[10]

Access to data and resources from the NEEAR water studies afford a unique opportunity to classify beach-goers' exposure to sand using: 1) self-reported survey results from detailed questionnaires administered during the summer swimming seasons of 2003-2005 and 2007 and 2) measured concentrations of fecal indicator organisms in beach sand. A sand monitoring approach using a suite of health effects sand quality indicators (i.e., *Enterococcus*, *Bacteroides*, *B. thetaiotaomicron*, and F^+-specific

coliphage) balances sensitivity and specificity to capture the variability of point source sewage contamination in wet beach sand at Fairhope Beach, AL and Goddard Memorial State Park Beach, RI. To the best of our knowledge this is the first study to fully investigate the association between exposure to beach sand and health outcomes and also to explore whether increasing levels of fecal indicators in sand increases beach-goers' risk of illness.

III. STATEMENT OF STUDY QUESTIONS

A. Study questions

1) Is there an increased risk of illness among people in contact with beach sand (digging in sand or building sandcastles; buried in sand) compared to those who are not in contact with beach sand (including among a subgroup of children ≤10 years of age)?
2) Is there an increased risk of illness among people in contact with beach sand with higher daily average levels of fecal indicator organisms present (*Enterococcus*, *Bacteroides*, *B. thetaiotaomicron*, and F^+-specific coliphage) compared to those in contact with beach sand with lower daily average levels of fecal indictor organisms?
3) Is there an increased risk of illness among people in contact with beach sand with higher daily average levels of fecal indicator organisms present compared to those who are not in contact with beach sand?

B. Hypotheses

1) There is an increased risk (incidence proportion) of illness among people in contact with beach sand compared to those who are not in contact with beach sand – including among a subgroup of children ≤10 years of age.
2) There is an increased risk (odds) of illness among people in contact with beach sand with higher daily average densities of fecal indicator organisms present

compared to those in contact with beach sand with lower daily average densities of fecal indicator organisms present.

3) There is an increased risk (odds) of illness among people who are in contact with beach sand with higher daily average densities of fecal indicator organisms present compared to those who are not in contact with beach sand.

C. Specific aims

Using data from the 2003-2005, and 2007 NEEAR water studies, we aim to:

1) Measure associations between self-reported contact with sand (e.g., digging in the sand or building sandcastles; being buried in the sand) and the risk (incidence proportion) of self-reported symptoms and illness during the 10-12 days following exposure — including a subgroup analysis among children ≤10 years of age.

Using data from the 2007 NEEAR water studies, we aim to:

2) Evaluate associations between daily average concentrations of fecal indicator organisms (*Enterococcus*, *Bacteroides*, *B. thetaiotaomicron*, and F^+-specific coliphage) in wet sand and illness risk (odds) during the 10-12 days following sand contact activities.

IV. METHODS

A. Overview of methods

1. NEEAR water study

The National Epidemiological and Environmental Assessment of Recreational (NEEAR) water study is a large prospective cohort study of beachgoer health. The NEEAR water study is designed and funded by the United States Environmental Protection Agency (EPA) and implemented through collaboration with the Centers for Disease Control and Prevention (CDC). The NEEAR water study was conducted during the 2003-2005 and 2007 summer swimming seasons at seven beaches across the United States. During 2003 and 2004 four Great Lakes freshwater beaches were sampled. The Great Lakes beaches included West Beach, Indiana Dunes National Lakeshore in Porter, Indiana; Huntington Beach in Bay Village, Ohio; Silver Beach in St. Joseph, Michigan; and Washington Park Beach in Michigan City, Indiana. Huntington Beach is on Lake Erie and the other beaches are on Lake Michigan. In 2005 a marine beach, Edgewater Beach in Biloxi, Mississippi, was sampled. In 2007 two marine water beaches were sampled: Fairhope Beach in Fairhope, Alabama and Goddard Memorial State Park Beach in Warwick, Rhode Island. Sampling and analysis of water for fecal microbial indicators was performed during 2003-2005 and during 2007, however, sampling and analysis of beach sand was performed during 2007 only.

Beach selection for the NEEAR water study was an important first step for collecting data. A set of criteria was outlined that fulfilled the requirements of the BEACH Act of 2000 while also maintaining cost-effectiveness. Criteria for beach selection were as follows: 1) must have been a coastal beach as outlined by the BEACH Act of 2000; 2) must have been an officially designated recreational area near a large population center; 3) must have had a large attendance (e.g., 300 - 400 swimmers/day); 4) the age range of the swimmers must have been broad (i.e., including children, teenagers, and adults); 5) the beach generally had to meet state or local water quality standards with a range of concentrations of fecal indicator bacteria (FIB); 6) the beach must have been contaminated by an identified human source of pollution (point-source); and 7) the swimming season must have been at least 90 days long.

For each beach, packets of informational materials were developed and sent to state and local health officials and regional EPA offices to inform them of the beach selection in their area, the project's purpose, and the intent to enlist support for the project. If beach managers were interested in participating, a site visit was made months prior to the swimming season to meet local parties and obtain preliminary planning and data collection information. This included: 1) GIS mapping of the beach and potential point source contamination; 2) daily bather load/usage data; 3) historical seasonal usage data to plan enrollment activities; and 4) mapping of access points and weather patterns for planning enrollment activities.

During the 2003-2005 and 2007 rounds of the NEEAR water study EPA collected data on several fecal indicators of beach water quality. EPA has completed studies of the relationship between these fecal indicators of water quality and gastrointestinal (GI)

illness. EPA is continuing preliminary analyses of associations between exposure markers for sewage in bathing water and health outcomes (including GI illness, diarrhea, upper respiratory illness (URI), urinary tract infection, skin rash, eye ailment, earache, and infected cuts) among beachgoers. These illnesses were selected because they have been shown to be health concerns of exposure to fecal contamination.

This research has been approved by the UNC Public Health-Nursing institutional review board (IRB) (IRB Study #07-0769) and the Centers for Disease Control and Prevention institutional review board (CDC IRB Protocol #3544) and makes use of data collection activities of the EPA NEEAR water study. This research involves: 1) secondary de-identified data of participant responses to questionnaires administered during the NEEAR water study; 2) collection of beach sand samples during the NEEAR water study; and 3) measurement of concentrations of fecal indicator organisms (i.e., *Enterococcus*, *Bacteroides*, and F^+-specific coliphage) in collected beach sand samples. The NEEAR water study questionnaire was for use during the 2004, 2005, and 2007 rounds of the NEEAR water study. Environmental sand sampling was performed at 8:00 am each day before the baseline enrollment questionnaire was administered to participants of the NEEAR water study.

The purpose of these two phases of research is to investigate potential associations between human contact with beach sand and health effects (due to exposure to pathogens of fecal origin in beach sand). The first phase involves a secondary data analysis of 2003-2005 and 2007 NEEAR water study data. Secondary data include participant self-reported contact with beach sand and self-reported physical symptoms

and illness after 10-12 days of follow-up. Associations were evaluated between contact with sand and the incidence of illness 10-12 days after beach sand contact activities.

During the second phase of research, beach sand samples were collected at Goddard Memorial State Park Beach, RI and at Fairhope Beach, AL during the 2007 NEEAR water study. Beach sand samples were analyzed to quantify concentrations of the following fecal indicators: *Enterococcus* (CFU) using culture methods (EPA Method 1600) [151] and a previously described and validated quantitative polymerase chain reaction cell equivalent (qPCR CCE) method [9], *Bacteroides* (qPCR CCE), *B. thetoiotaomicron* (qPCR CCE), and F^+-specific coliphage (EPA Method 1601). Investigators at US EPA NCER in Cincinnati, Ohio and local laboratories in Alabama (Severn Trent Laboratories, Inc.) and Rhode Island (BAL Laboratories, Inc.) completed *Enterococcus, Bacteroides, and B. thetaiotaomicron* analyses. F^+-specific coliphage analyses were completed at the Environmental Health Microbiology and Virology Laboratory at the University of North Carolina at Chapel Hill. Associations between laboratory results of fecal indicator organism measurements in beach sand and self-reported health outcomes were evaluated adjusting for covariates of interest. During both phases of research, sub-group analyses were conducted to examine the potential increased susceptibility of children (≤10 years of age) to illness following recreational contact with beach sand.

Data collected at Fairhope Beach, Alabama and Goddard Memorial State Park Beach, Rhode Island during the 2007 summer swim season included environmental and beach sand measures (*e.g.*, meteorologic conditions, beach conditions, and *Enterococcus*, *Bacteroides*, and F^+-specific coliphage concentrations in beach sand) on days of participant recruitment for the 2007 NEEAR water study. Self-reported data was

abstracted from NEEAR water study participant responses to a detailed questionnaire administered via in-person interview at baseline and at departure from the beach and via telephone interview after 10-12 days of follow-up. Measurement of fecal indicators in beach sand was performed at 8:00 AM each day of NEEAR water study participant recruitment between May 19, 2007 and September 2, 2007 (weekend days and holidays). Sand samples were collected at three transects along the beach that correspond to the water sample collection transects.

B. Study Design

1. Participant identification/sampling

    a. Source population: Beach enrollment and beach exit questionnaires

A prospective cohort study was used to enrolled participants over four summer swim seasons at four freshwater beaches (2003-2004) and two marine beaches (2005 and 2007). Interviewers attempted to approach all beach-goers between 11:00 A.M. and 5:00 P.M on the day of their visit (weekend days and holidays). Interviewers excluded unaccompanied minors (below 18 years) or those who could not speak English or Spanish. During the baseline interview, participants were asked about illnesses experienced during the 3 days prior to their visit to the beach. Upon leaving the beach, participants were interviewed to ascertain information about beach activities, including swimming and contact with beach sand. Ten to 12 days following the beach-exit interview all participants were interviewed by telephone to determine whether they developed enteric and/or non-enteric illness during the time since their beach visit. Demographic information, exposures, covariates, and physical symptoms and illness

were measured via a detailed questionnaire administered at baseline, departure from the beach, and after 10-12 days of follow-up. During 2003-2005 and 2007 interviewers performed the following:

1) Approached all beach-goers about participation in the beach study;
2) Enrolled participants on the beach and obtained verbal consent and obtained demographic information on the household members at the beach;
3) Interviewed enrollees as they left the beach to answer questions about their activities, including swimming habits and contact with sand during that beach visit;
4) Followed-up participants using a detailed health effects telephone questionnaire 10-12 days after their beach visit.

All beach-goers were approached on weekend days during the designated study period. An adult family member was approached for initial enrollment. After receiving verbal consent, eligibility was determined. Subsequently information on family make-up/membership, demographics, and baseline activities and illness were obtained. Follow-up contact information was also requested. After completion of the enrollment interview (at baseline), families were encouraged to visit project work sites near exits on the beach, when they were leaving, to complete the exit beach questionnaire. The information collected during the beach exit interview included the day's activities, including food and water consumption, water and sand exposure (extent, duration, frequency, and location of swimming), and other covariates. The questionnaire obtained individual level information on health status and potential confounders such as age, sex, race, Hispanic

status, housing characteristics, family characteristics, and behaviors. A low cost incentive was offered following completion of the beach questionnaire.

b. Identification of participants with physical symptoms and illness

Ten to 12 days after the family's beach visit, an adult caregiver who participated in the enrollment/baseline and beach exit interviews (preferably the original one interviewed the day of beach enrollment) was asked a series of questions about family members' health status and potential burden of illness since the beach exit interview. Questions covered common physical symptoms of enteric and non-enteric illnesses (gastrointestinal, diarrhea, upper respiratory, skin rash, ear, eye, cut/wound infections) and, if possible, determined the duration and frequency of any reported illness. A low cost incentive was given following completion of the telephone questionnaire.

2. Methods

a. Classification of exposure

1. Exposure of interest

We considered two primary exposures of interest. The first was self reported contact with beach sand which was measured by two questions on the enrollment-day beach exit survey. The first question covered digging in the sand or playing in the sand and the second question asked whether participants had their body buried in the sand. Multiple survey questions on participant contact with beach sand allowed us to evaluate two methods of defining exposure. Self-reported sand contact activities were reported as binary (0 = No; 1 = Yes). The exposure measure during the second phase of research

was the concentration of fecal indicators (*Enterococcus*, *Bacteroides*, *B. thetaiotaomicron*, F^+-specific coliphage) measured in beach sand samples. Several methods of exposure classification were considered for the fecal indicators data: classification as a continuous variable, binary variable, and ordinal categorical variable.

2. Exposure period

The exposure period was defined as the period of recreational activity at the beach between the baseline/enrollment and beach exit interviews. The enrollment interview was conducted during the morning when participants arrived at the beach and the exit interview was conducted after recreational activities were completed as participants were leaving the beach (the same day of enrollment). During the enrollment and exit interviews participants were asked about exposures they experienced during the 3 days prior to enrollment and on the day of enrollment, respectively. Exposures that occurred 3 days before the day of enrollment included activities such as swimming at a pool, swimming at another beach, swimming at the same beach, or eating raw or undercooked eggs, red meat, fish, or shellfish. These exposure activity questions were repeated during the telephone follow-up questionnaire conducted 10-12 days after participants left the beach.

3. Beach sand exposure measurement

For the first phase, beach sand exposure was measured by participant self-report of contact with beach sand during the 2003, 2004, 2005, and 2007 NEEAR water studies. Upon leaving the beach in 2003, 2004, and 2005, NEEAR water study participants were asked to self-report if they:

1) Had been digging in the sand or building sand castles, or
2) had their body buried in the sand.

During the 2007 NEEAR water studies beach sand exposure questions were expanded. Upon leaving the beach, participants were asked the following instead of 1) and 2) above:

1) Did you engage in any of the following activities while at the beach today?
    a. Collecting sea shells, rocks, feathers, etc?
    b. Digging in sand or building sand castles?
    c. Had your body buried in sand?
        1. (If YES to 1.b., or 1.c.) Did you get any sand in your mouth?
            a) After digging in the sand, or building sand castles…did you eat anything with your hands? (not necessarily at beach)?
            b) After digging in the sand, or building sand castles did you wash your hands before eating (washing of hands may include the use of a personal water-free hand sanitizer)?

Participant answers to this series of questions were coded as: Yes = 1, No = 2, Refused = 7, or Don't Know = 8.

During phase two, self-reported data from the exit interview (as described above) was combined with results from the fecal indicator beach sand sampling effort. Beach sand sampling was conducted prospectively during the 2007 NEEAR water study to measure fecal indicator concentrations (*Enterococcus*, *Bacteroides*, *B. thetaiotaomicron*, and F^+-specific coliphage) in beach sand along three transects at Goddard Memorial State Park Beach, RI and Fairhope Beach, AL. This provided a continuous measure of sand

exposure and allowed us to perform an evaluation of associations between fecal indicator concentrations in sand and NEEAR water study participant self-reports of illness (including GI illness, diarrhea, upper respiratory illness, eye ailment, earache, skin rash, and infected cuts/wounds).

In the following sections, we describe various approaches to the distribution of sand samples, method of sand sample collection, method of measuring fecal indicators (in sand samples at beaches), and statistical methods to evaluate sand exposure as well as associations between fecal indicators in sand and health outcomes.

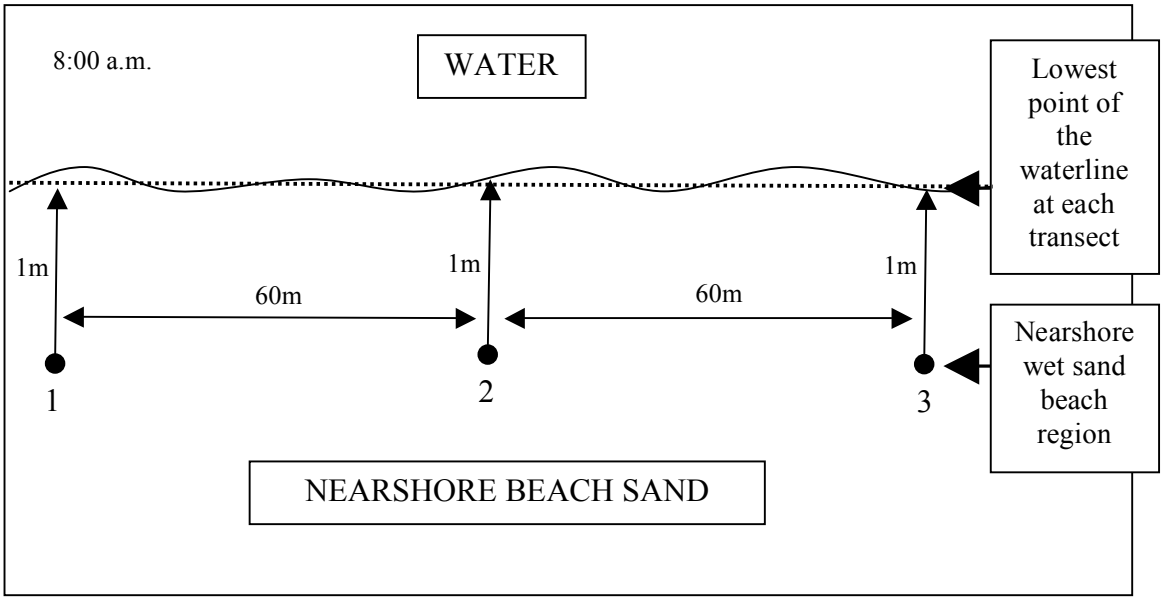
4. Consideration of the distribution of sand samples

The sand sampling strategy for the proposed research was based on previously published approaches from the scientific literature.[152-154] There were many factors to consider in developing a sand sampling strategy (e.g., distance from the waterline, depth, and timing of sample collection). Past studies have involved complex sand sampling strategies; however, that was not the focus of this research. Cost was a constraint on the spatial and temporal frequency of sand samples that could be collected. Available resources allowed for collection of 3 beach sand samples during the 2007 NEEAR water studies. It was important to consider several methods to collect these samples to identify the approach that would balance costs and allow us to adequately evaluate associations with health effects.

Sufficient financial resources were not available to collect sand samples at varying distances from the waterline (i.e., nearshore vs. backshore sand of the beach), nor to collect samples at varying depths or varying time points during the day. We employed a sand sampling strategy involving collection each weekend day (i.e., Saturday and

Sunday) of the 2007 NEEAR water study at 8:00 a.m., at a distance of 1 m from the lowest point of the waterline (see Figure 1), at a depth of at least 8 cm (including the Independence Day and Labor Day holidays). Sand was collected along 3 transects (the same 3 transects for water sampling) using a soil auger at distance of 1 m from the lowest point of the waterline, at a depth of at least 8 cm (see Figure 1). This method accounted for the movement of the tides because the sand sample collection point (lowest point of the water line) would move along with the tides (i.e., our sampling point followed the lowest point of the water line and the sample was taken 1 m from that point).

Figure 1. Distribution of wet sand sampling points (1-3) along three transects approximately 60 m apart (not drawn to scale).

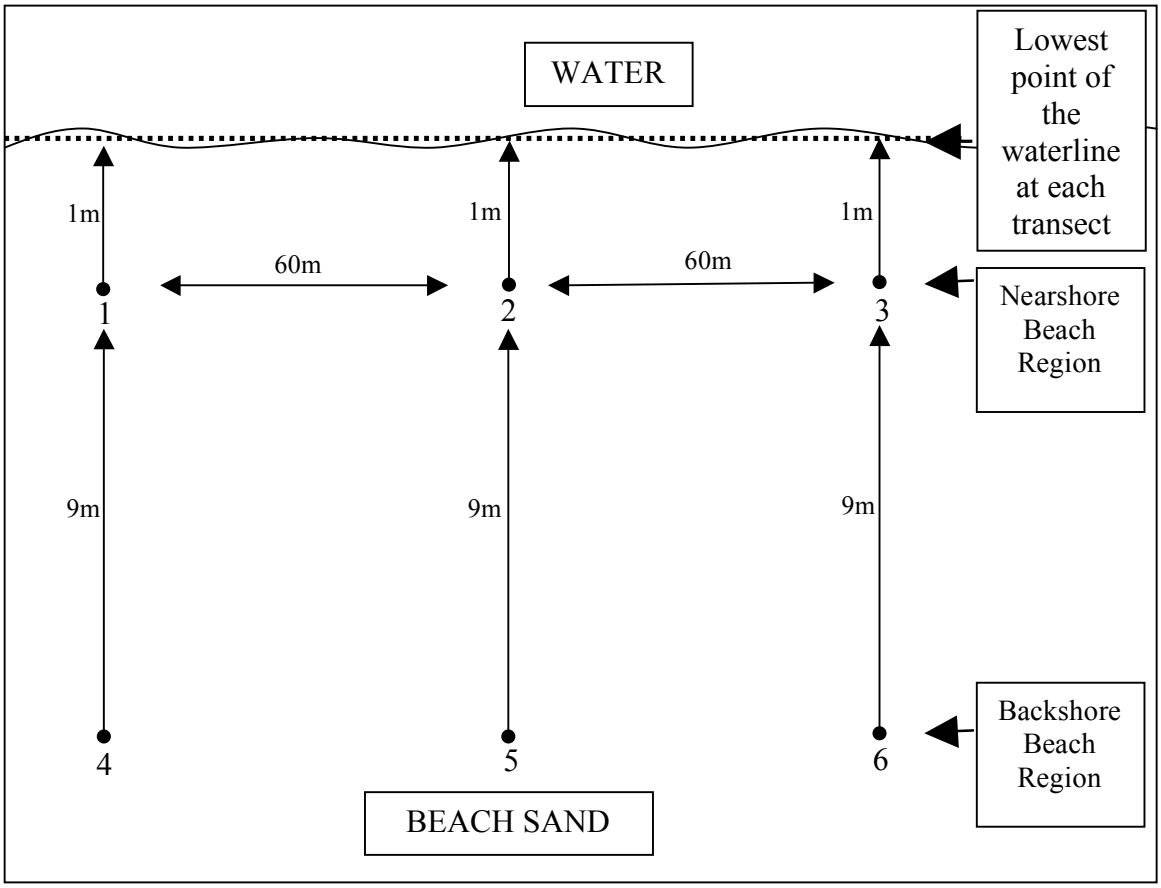


The sand sampling approach was evaluated after a local laboratory fecal indicator results from a dry run were reviewed. Laboratory fecal indicator results from our sand sampling approach were also reviewed during the first week of enrollment of the 2007 NEEAR water study (at Fairhope Beach, AL). During the initial weeks of the study, several sand sampling factors listed above could have been changed (e.g., distance from waterline, depth below surface, and time of day); however, given the satisfactory review

of results from the local laboratory (Severn Trent Laboratories, Inc. Mobile, AL) after the dry run and first week of sampling during enrollment, the sand sampling approach was not modified.

For completeness, a presentation of an alternate sand sampling approach is presented (see Figure 2). For example, 2 samples could have been collected—one sample 1 m from the waterline (wet sand) and one ~10 m from the waterline (to capture variability in fecal indicators in wet sand vs. dry sand) (see Figure 2). Briefly, alternate approaches could also have involved sampling sand: (1) at varying depths below the surface (e.g., 8 cm, 50 cm, 1 m, 2 m, etc.); (2) at varying times throughout the day (8:00 a.m., 11:00 a.m., and 3:00 p.m.); and (3) at varying distances from the waterline (at 1 m to capture nearshore wet sand quality vs. at 10 meters to capture backshore dry sand quality). Figure 2 shows an example of an alternate sand sampling approach where samples could have been collected at varying distances from the waterline. This would have allowed for comparisons of sand quality in the nearshore region (i.e., wet sand) compared to the backshore region (i.e., dry sand). Although several studies have used this approach to compare sand quality at varying distances from the waterline, we will not adopt this approach based upon the assumption that sewage discharges from nearby municipal outfalls will more likely impact the nearshore wet sand region as opposed to the backshore dry sand region. The sampling approach was not changed after the dry run and initial weeks of the 2007 NEEAR water study. An alternate sand sampling approach could have been considered during the 2007 NEEAR water study, however, approach outlined in Figure 1 was adopted for this research.

Figure 2. Alternate sample distribution example for wet nearshore sand (locations 1-3) vs. dry backshore sand (locations 4-6) along six transects 60 m apart (not drawn to scale).



The sand sampling strategy illustrated in Figure 2 served as an acknowledgement of alternative sand sampling strategies. Six sand samples could have been collected once each day (8a.m.) at a depth of at least 8 cm, 1 m from the waterline (wet sand) and six sand samples could be collected once each day (8a.m.) at a depth of at least 8 cm, 10 m from the waterline (dry sand). The goal of the sand sampling approach was to capture samples representative of sand quality in the nearshore wet sand region of the beach. All sand samples were assayed for fecal indicators (*Enterococcus*, *Bacteroides*, *B. thetaiotaomicron*, and F^+-specific coliphage) using previously described and validated methods.[92,155-157] Culture-based concentrations of *Enterococcus* in sand were assayed by

Severn Trent Laboratories, Inc in Mobile, Alabama. Investigators at the US EPA NCER in Cincinnati, Ohio assayed molecular *Enterococcus*, *Bacteroides*, and *B. thetaiotaomicron* in sand. Culture-based F^+-specific coliphage was assayed at the Environmental Health Microbiology and Virology Laboratory at the University of North Carolina at Chapel Hill.

5. Beach sand sample collection

We collected 3 beach sand samples along with the 8:00 a.m. water samples each weekend day. The sand samples were collected 1 meter from the lowest point of the water level (when the waves receded to their lowest point from the shoreline) at the same 3 transects where water samples were collected. The sand was wet. If the sand was not wet at 1 meter from the waterline, the sand collection location was moved the shortest possible distance toward the water to a location where the sand was wet. The actual distance from the water was recorded if this occurred. Global Positioning System (GPS) readings of the actual sand collection locations and a photo of the sample collection sites were taken.

Sand samples were collected with a 2.25-inch diameter stainless steel soil auger with sterile, 2 inch x 12 inch plastic liners (AMS, American Falls, Idaho, or the equivalent). The auger was pushed into the sand at least 8 inches. If polypropylene liners were not available, the liners were sterilized using ethylene oxide or 70 % ethanol. Liners containing the sand samples were capped at both ends, placed in zip-lock plastic bags labeled using a simplified version of the usual alpha-numeric system (see below), and transported to the laboratory on ice. Samples were stored in a refrigerator at 4 °C until analyzed.

6. Beach sand sample analysis for fecal indicators

In the laboratory, sand samples were aseptically transferred to sterile wide-mouth polypropylene bottles (500 ml or 1- liter, depending on the quantity of the sand), and labeled using the simplified version of the usual alpha-numeric labeling system.

a. *Enterococcus*

For each sand sample, 75 grams of sand was aseptically weighed out in a sterile, pre-tared, wide-mouth 500-ml bottle (using sterile spatulas), and 300 ml of Standard Methods[73] phosphate-buffered rinse/dilution water was measured with a sterile graduated cylinder and was added to each bottle. Each bottle was vigorously shaken 50 times. Immediately after shaking, some of the contents of the bottle were poured into two sterile 50-ml, disposable centrifuge tubes (Corning 430829 or the equivalent) and filled to the 50-ml mark. The supernatant was removed from the centrifuge tubes using a sterile pipette and placed in a sterile 100-ml polypropylene bottle for subsequent analysis by Method 1600 and the quantitative polymerase chain reaction cell equivalent (qPCR CCE) method.[136,151] This molecular method was also applied to quantification of *Bacteroides* and *B. thetaiotaomicron* assays.

The accuracy of the 50-ml mark on the disposable tubes was checked before the dry run by randomly choosing 5 tubes from the package, weighing each of the 5 tubes, and recording the weights. After 50 ml of distilled water was measured with a graduated cylinder and poured into each of the tubes, the tubes were again weighed. The position of the water meniscus was observed with reference to the 50-ml mark on the tubes. In addition, 5 randomly chosen, preweighed tubes were filled with distilled water so that the meniscus touched the top of the 50-ml line. The tubes were weighed again to determine

the weight of the water by difference. If the mark was accurate, the weight of the distilled water was close to 50 grams. All results were recorded and copies were sent to investigators at EPA.

During the dry run, aliquots of 10 ml and 1 ml of each undiluted sand extract and 1 ml of the $10^{-1} – 10^{-6}$ dilutions of each extract in phosphate-buffered dilution water were analyzed by EPA Method 1600 for *Enterococcus*. The number of filtrations for the actual study was reduced after the normal range of concentrations in sand was determined during the dry run. Three 20-ml aliquots of each sample were filtered, and the filters will be frozen, as described in the QPCR CCE Method, during the dry run. The sand extraction method described above and the volumes used for both tests were adjusted, depending on the normal range of concentrations of *Enterococcus* in the extracts observed during the dry run. The laboratory and contractor obtained EPA's approval before they changed the protocol or volumes analyzed.

In addition, the pH of each extract was taken and recorded, and a 25-gram portion of each sand sample was dried at 100 degrees C for several days to a week in a preweighed container. After the samples were dry, the containers were weighed again to determine the dry weight of the sand samples by difference. Leftover sand samples, the bottles of the sand-buffer slurry, and extracts were stored in the refrigerator until all the results had been obtained with all *Enterococcus* Method 1600 tests.

b. *Bacteroides* and *B. thetaiotaomicron*

At the contract laboratory a separate volume of the sand-buffer slurry solution was membrane filtered. The extract on the filters was frozen and the filters were sent on

dry ice to the contract laboratory to be assayed by QPCR CCE method for *Bacteroides* and *B. thetaiotaomicron* using previously validated methods.[75,158-160]

c. F^+-specific coliphage

After the needed sand sample volume was removed for the *Enterococcus* and *Bacteroides* analyses, ≥250 g of sand from the leftover sand sample was aseptically weighed and transferred to a new wide-mouth polypropylene sand sample bottle (500 ml or 1- liter, depending on the quantity of the sand or similar suitable sterile container). At least 250 g of sand was weighed and transferred. The amount weighed and transferred (in grams) was labeled on the new sand sample bottle. The new sand sample bottle was labeled with the sample ID that was assigned with the simplified version of the usual alpha-numeric labeling system. This was repeated for each sand sample that was collected on each weekend day. The sand sample bottles were placed in a small plastic cooler (e.g., cardboard-lined styrofoam shipping cooler) containing dry ice or several blue ice packs and shipped via FedEx priority overnight. The sand samples arrived at the EPA Human Studies Division in Chapel Hill at the latest on Tuesday mornings (10:00 a.m.) following sample collection on Saturday and Sunday each previous weekend. A 74 hour holding time for the Saturday 8:00 a.m. sand sample was not exceeded. All samples were analyzed using EPA standard Method 1601: Male-specific (F^+) and somatic coliphage in water by two-step enrichment procedure [89].

7. Beach sand sample alpha-numeric system

To avoid confusion and duplicate sample numbers we used the following simplified alphanumeric (9-digit) scheme for sand sample ID numbers: S-MMDD-

NPPXX. Where: S stood for Sand; MMDD was the date of the sample collection; MM was the numeric month and DD was the day, *e.g.*, 0614 for June 14; N was the transect location at the beach (1 – 3, left to right when facing the water); PP was the analytical method number, using either 01 = Membrane Filter Method 1600 or 02 = QPCR Method; XX was the planned time of day for the sample collection using 08 = 8:00 a.m., 11 = 11:00 a.m., and 15 = 3:00 p.m. (8:00 a.m. was the only planned time for sand sample collection).

8. Beach sand sample data collection activities

Each Saturday and Sunday of participant recruitment during the 2007 NEEAR water studies the collection of a total of three sand samples was coordinated. One sample was collected at each of the 3 transects where water samples were collected. The sand samples were collected 1 m from the lowest point of the waterline at a depth of at least 8 cm to ensure that the sand was wet. Investigators at the EPA performed an initial site visit to each beach and also made follow-up visits each weekend of participant recruitment throughout the summer swim season to ensure proper collection of beach sand samples and proper laboratory processing of beach sand samples.

Weekend day beach sand monitoring followed the sample collection and laboratory analysis procedures described above. EPA trained contractors and beach field monitoring staff. The contractor and beach field monitoring staff training were an essential to ensure that all beach sand samples were collected correctly and with the appropriate care each morning of participant recruitment. Training included review of written instructions covering beach sand sample collection activities, including sample collection equipment operation and the proper handling and storage of collected sand

samples. Each day of participant recruitment of the 2007 NEEAR water study beach sand samples were collected (weather permitting). Lightning at the time of sample collection suspended sand sampling activities for that day. Sand sample collection was scheduled at 8:00 a.m. and preceded participant the enrollment and baseline interview and enrollment day exit interview.

b. Classification of outcome

1. Measurement of physical health symptoms and illness

NEEAR water study participants were interviewed at enrollment and asked to self-report physical health symptoms and illness at baseline and during the 3 days prior to enrollment. As they departed from the beach at the end of the day of enrollment, participants were asked to report beach activities and were also asked for permission to conduct a follow-up telephone interview. Participants who agreed were contacted by telephone 10-12 days following their beach exposure and asked to self-report if they had experienced any of the following symptoms or illnesses since their exit interview on the day of beach recreation: (1) gastrointestinal (GI) illness; (2) diarrhea; (3) upper respiratory illness; (4) eye ailments; (4) earache; (6) skin rash; or (7) infected cuts or wounds. Participants' self-reported answers to a series of questions about the occurrence of physical symptoms and illness were coded as: Yes = 1, No = 2, Refused = 7, or Don't know = 8.

2. Physical health symptoms and illness data

Secondary health outcome data was abstracted from the 2007 NEEAR water study using questionnaire results from the NEEAR Water Studies (2003-2005 and 2007). Data included information about self-reported physical health symptoms, illness, and beach exposures. This secondary dataset contained de-identified self-reports of physical health symptoms and illness measured once at baseline (to and again at 10-12 days of follow-up after enrollment day beach activities (to distinguish between prevalent and incident illness).

Secondary data included binary self-reported: 1) GI illness; 2) respiratory illness (upper and lower); 3) earache; 5) eye ailment (watery eyes, irritation, or infection); 6) rash; 7) infected cuts or wounds, and 8) potential confounders (e.g., age, race, sex, beach site, swimming status). Symptoms and illness were assessed via in-person questionnaire at baseline and three days prior to enrollment (including use of prescriptions, diagnosis of pre-existing medical conditions, etc.). It was important to have recent medical history and physical symptoms data to be able to document a change in health status during the follow-up period and also to identify potential exclusion criteria. For example, persons who reported GI illness in the three days prior to enrollment were excluded from analysis of GI illness, but were eligible for analyses of other illnesses. The secondary data set from the 2003-2005 and 2007 rounds of the NEEAR water study allow us to capture information related to frequency and duration of health symptoms (e.g. "What day did symptoms start?" and "How many days did the symptoms last?"). Surveys at baseline and after 10-12 days of follow-up allow us to differentiate between pre-existing outcomes and incident outcomes. Secondary data including frequency (number of symptom

episodes in past two weeks) and duration (how many days symptoms lasted) were abstracted for the following physical health symptoms and illnesses:

1. GI illness, defined as any of the following: a) Diarrhea (3 or more loose stools in a 24 hour period), b) vomiting, c) nausea and stomachache;
2. Diarrhea alone (3 or more loose stools in a 24 hour period);
3. Upper Respiratory illness (URI), defined as any two of the following: sore throat, cough, runny nose, cold, fever;
4. Skin Rash;
5. Eye ailment, defined as either eye infection or watery eye;
6. Earache;
7. Infected cuts or wounds.

3. Data analysis methods

a. Data checking

A preliminary review of the environmental exposure (*e.g.*, beach sand samples, environmental beach conditions, and meteorologic conditions) and health outcome secondary data of physical symptoms and illness from all rounds of the NEEAR water study were performed to check for outliers and implausible data points. Errors in data were evaluated and if deemed actual errors by comparisons with responses of other participants and based upon substantive reasoning, then they were deleted from the data set. Data sets were also cleaned and evaluated for missing values. Beach sand measurements of fecal indicators (*Enterococcus*, *Bacteroides*, and F^{+}-specific coliphage)

were recorded as continuous measures in data sets on secure servers at the EPA, Research Triangle Park, NC.

b. Overview of data analysis

We examined the association between beach sand exposure and physical health symptoms and illness using prospective data collected during the NEEAR water study (2003-2005 and 2007) from 7 recreational beach sites across the U.S. Data analysis was completed in two phases. First, we evaluated the association between self-reported exposure to beach sand [digging in the sand or building sand castles; burying one's body in the sand] and the incidence of self-reported physical health symptoms and illness during 10-12 days follow-up (Study Question 1, Hypothesis 1, and Specific Aim 1). Next, we evaluated the association between exposure to fecal indicators (*Enterococcus*, *Bacteroides*, *B. thetaiotaomicron*, and F^+-specific coliphage) measured in beach sand samples and the incidence of self-reported health symptoms and illness during 10-12 days of follow-up (Study Question 2, Hypothesis 2, and Specific Aim 2).

There were 2 hypotheses that corresponded to the 2 phases of research. For phase 1, we hypothesized that among people who were exposed to beach sand the incidence proportion of illness would be higher than the incidence proportion of illness among people unexposed to beach sand (including among a subgroup of children ≤10 years of age). For phase 2, we hypothesized that among people who were exposed to beach sand with higher levels of fecal indicator organisms, the incidence proportion of illness would be higher than the incidence proportion among people who were exposed to beach sand with lower levels of fecal indicator organisms (including among a subgroup of children ≤10 years of age).

The primary outcomes were self-reports of physical symptoms and illness, measured on a binary scale with 1 being YES to a physical symptom and 2 being NO to a physical symptom. Physical symptoms were grouped into 7 outcomes (illnesses). We considered the following: (1) GI illness, 2) Diarrhea, (3) Upper Respiratory illness (URI); (4) Rash; (5) Eye; (6) Earache; and 7) Infected cuts or wounds.

To exclude those with prevalent illness, participants who were ill in the 3 days prior to their beach visit were excluded for the outcome with which they were afflicted. For example, a subject who reported GI symptoms at baseline was excluded for the GI analysis, but was eligible for other outcomes. We also examined different definitions of GI illness, including diarrhea alone (three or more loose stools in a 24 hour period).

c. Data analysis (Specific Aims 1 & 2)

For phase 1 (Specific Aim 1) the hypothesis was that the incidence proportion of illness would be higher among those who were exposed to beach sand compared to the incidence proportion of illness among those unexposed to beach sand (including among a subgroup of children ≤10 years of age). For phase 2 (Specific Aim 2) the hypothesis was that among people who were exposed to beach sand with higher levels of fecal indicator organisms, the incidence proportion of illness would be higher than the incidence proportion among people who were exposed to beach sand with lower levels of fecal indicator organisms (including among a subgroup of children ≤10 years of age).

Critical covariates for this analysis included age, sex, race/ethnicity, swimming, beach, contact with animals, contact with other persons with diarrhea, number of other visits to the beach, any other chronic illnesses (GI, skin, asthma), eating food while at the beach, eating raw or undercooked meat since the time of the beach interview, and eating

raw or undercooked eggs since the time of the beach interview. For respiratory and skin outcomes, the use of insect repellent and sun block were also considered. A variable was also created to control for a large festival that took place at Silver Beach, drawing over 17,000 visitors directly adjacent to the beach. A number of covariates, especially swimming status and beach site, were considered potential important effect measure modifiers of exposure-illness associations.

We considered using log-linear binomial regression to model the association between beach sand quality measures and health effects, however, a small sample size led to problems with model convergence. We therefore used logistic regression models to estimate the association between the incidence of illness and *Enterococcus* qPCR CCE/g, *Enterococcus* Method 1600 CFU/g, *Bacteroides* qPCR CCE/g, and F^{+}-specific coliphage PFU/g, respectively in sand: (1) among those who had contact with the sand; and (2) among all participants with those who did not have contact with sand as the reference category. Generalized linear models (GLM) using an identity link and a binomial error structure were also considered to estimate the attributable risk (contact with sand minus no-contact with sand), which we also will refer to as sand-associated illness. We considered that the robust variance estimates, or the "sandwich" estimator of variance, was used in all models to account for the non-independence resulting from the household-cluster sample.

Incidence proportion ratios (IPR) were used to estimate the association between exposure data (*i.e.*, self-reported contact with sand) and follow-up health outcome data (self-reported illness). At the start of analysis a 5% change-in-estimate of IPRs and backwards elimination approach was employed. Potential effect measure modifiers were

evaluated using stratification and the Breslow-Day test of homogeneity. All analyses were completed using SAS version 9 © (SAS Institute Inc., Cary, NC, USA), Stata version 9.2 © (StataCorp LP, College Station, TX, USA).

d. Statistical power calculation 1

This study involved hypothesis testing and the study size was fixed. We estimated power. The first hypothesis was was that among people who were exposed to beach sand the incidence proportion of illness would be higher than the incidence proportion of illness among people unexposed to beach sand.

For this power calculation we assumed that alpha was 0.05 and that our study size, based on data from a preliminary study by Wade et al, (2006) and from unpublished data, was 20,436. We were trying to estimate beta. To examine statistical power for binary outcome variables, we used the formulae given by Clayton and Hills (1996), Friedman et al., (1998), and Selvin (1996). To simplify the power analysis, we considered the simple problem of comparing two levels of exposure (exposed and unexposed) with 8,975 participants in the exposed group (those digging in the sand) and 11,461 participants in the unexposed group (those not digging in the sand) as observed by Wade et al, (2006) in a preliminary study and from unpublished data. This power calculation reflected phase one plans for binary exposure categories. We considered other exposure classification methods. In phase two of the proposed research, exposure was represented by a continuous index (e.g. CFU, qPCR CCE, or PFU per g of sand). In addition, we assumed that individuals are independent. We assumed two-sided tests and a Type I error rate (significance level) of 5%.

Considering a binary outcome variable, onset of symptoms and again using unpublished data data provided by Tim Wade (EPA), we defined "onset" for a given symptom as a "Yes" self-report on a binary scale. Among the seven illnesses and physical symptoms studied, incidence ranged from 7.4% for GI illness, to 0.4% for infected cuts, with an average incidence across all symptoms of 3.1%. For the purposes of power analysis, we considered the incidence distribution of each of the illnesses studied by Wade et al, (2006) and from unpublished data sets.

Table 1. Smallest Detectable Incidence Proportion Ratio

Incidence of illness and symptoms (from preliminary data)	Power	
	80%	90%
7.4% (GI illness)	1.15	1.17
5.8% (Upper respiratory illness)	1.17	1.20
3.0% (Eye irritation)	1.25	1.29
2.7% (Rash)	1.26	1.31
1.5% (Earache)	1.37	1.45
0.6% (Urinary tract infection)	1.66	1.81
0.4% (Infected cut)	1.88	2.10

Table 1 shows the smallest detectable incidence proportion ratio (IPR) across the distribution of incidence for each illness, for 80% and 90% power. For GI illness the study has good power to detect an IPR of 1.17. In general, the study has good power to detect IPRs around 1.25.

e. Statistical power calculation 2

For this power calculation we focused on children as a sub-group (≤10 years of age). The study involved hypothesis testing and the study size was fixed. We estimated power. The hypothesis was that, among a subgroup of children ≤10 years of age who are

exposed to beach sand, the incidence proportion of illness was higher compared to the incidence proportion among those who were not exposed to beach sand.

We assumed that alpha was 0.05, that our sub-group analysis study size was 4,712 based on unpublished data from Tim Wade and that we were estimating beta. The 4,712 individuals consisted of children (10 years of age or younger) from each of the five 2003-2005 NEEAR water studies beach sites. To examine statistical power for binary outcome variables, we used the formulae given by Clayton and Hills (1996), Friedman et al (1998), and Selvin (1996). To simplify the power analysis, we considered the simple problem of comparing two levels of exposure (exposed and unexposed) with 3,566 children in the exposed group (those digging in the sand) and 720 children in the unexposed group (those not digging in the sand) as observed by Wade et al., 2006 in a preliminary study and from unpublished data. This power calculation reflected phase 1 plans to consider binary exposure categories (other exposure classification methods were considered). In phase 2 of the proposed research, exposure was represented by a continuous index (*e.g.*, CFU or qPCR CCE *Enterococcus* concentration per g of sand). We assumed that individuals were independent. We assumed two-sided tests and a Type I error rate (significance level) of 5%.

Considering a binary outcome variable, onset of symptoms, and again using data from our preliminary study and unpublished data, we defined "onset" for a given symptom as a YES self-report on a binary scale. Across the seven illnesses and physical symptoms, incidence ranged from 8.6% for GI illness, to 0.2% for infected cuts, with an average incidence across all symptoms of 3.6%.

Table 2. Smallest Detectable Incidence Proportion Ratio		
Incidence of illness and symptoms among children (from preliminary data)	Power 80%	90%
8.6% (GI illness)	1.56	1.70
8.2% (Upper respiratory illness)	1.58	1.73
3.2% (Rash)	2.40	3.20
2.2% (Eye irritation)	3.61	8.00
1.8.% (Earache)	6.00	N/A*
0.7% (Urinary tract infection)	N/A*	N/A*
0.2% (Infected cut)	N/A*	N/A*

* Incidence of illness is too low to calculate 90% power.

We excluded participants with illness at baseline (prevalent cases). For the purposes of power analysis, we considered the incidence distribution of each of the illnesses studied by Wade et al, (2006). Table 2 shows the smallest detectable cumulative incidence proportion ratio (IPR) across the distribution of incidence for each illness, for 80% and 90% power. For GI illness among the sub-group of children ≤10 years old the study has good power to detect IPRs of around 1.56. Preliminary unpublished data and data from Wade et al, (2006) show that the study may not have good power to detect IPRs for illnesses with an incidence below 2.2%. In general, this power calculation demonstrated that the sub-group study had good power to detect IPRs around 1.7.

V. RESULTS

A. Contact with beach sand among beach-goers and risk of illness

1. Introduction

Recently, numerous studies of fecal contamination in beach sand have triggered interest among scientists, the news media, and the general public.[22,40-45,65,68,69,99,108,110,120,122,123,137,138,161] There is evidence that beach sand may harbor higher concentrations of fecal indicator organisms (microbes whose presence indicates the potential presence of fecal pathogens) than nearby bathing waters.[43-45,65,68,79,110,123] These studies have consistently showed high concentrations of fecal indicator organisms in beach sand and sediment during the summer swimming season and also throughout the year. Wheeler Alm et al., observed a mean summer abundance of *E. coli* and *Enterococcus* 3-38 times higher in the top 20 cm of wet-sand cores compared to levels in the water column at six freshwater bathing beaches on Lake Huron, MI.[43] In addition to fecal indicator organisms, several studies have detected pathogenic bacteria (*Pseudomonas aeruginosa*, *Salmonella*, *Shigella*, *Campylobacter jejuni*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, and *Vibrio harveyi*), viruses (adenovirus, norovirus, enterovirus, coxsackievirus types A16, B1, and B5, echovirus type 1, poliovirus type 2, hepatitis A virus), fungi (*Candida albicans* and dermatophytic fungi), and parasitic nematodes (*Toxocara canis*) in beach sand.[45,64,65,68-70,96,98,128]

The sources of high levels of fecal microbial pollution in beach sand are not clear. Some research attributed the source of the fecal pollution to municipal sewage treatment

plant discharges in close proximity to beaches; however, other studies attributed the source of fecal pollution to non-point sources such as urban runoff and/or warm-blooded domestic and wild animals.[43,68,110,111] Numerous studies found that the conditions in foreshore, nearshore, and backshore sand can favor the persistence, survival, and re-growth of *E. coli* and *Enterococcus* suggesting that elevated levels of these fecal indicator bacteria (FIB) in beach sand may represent autochthonous populations rather than impacts from sewage sources of contamination.[32,41,124] Conditions that favor the persistence, survival, and possible re-growth of autochthonous FIB in sand include increased protection from sunlight, buffered temperatures, more nutrient availability, reduced osmotic stress, cover from predation by other microorganisms, a large surface area for biofilm development, and higher moisture and organic content from wave swash.[32,45] Some studies suggest that conditions in nearshore wet sand may be more favorable for FIB survival than backshore dry sand,[32,33] while others suggest that dry sand conditions favor the ability of *E. coli* and other FIB to outcompete predators and survive as an autochthonous population.[124] It is not entirely clear which fecal microbial pollution sources initially populate the beach sand community. However, nearshore wet sand is more likely than backshore dry sand to be impacted directly by municipal sewage treatment plant discharges via wave swash.[40,41,43] It is also likely that nearshore wet sand (as well as backshore dry sand) is impacted by pollution from animal (gull, other bird, and domestic pet) and human (at beach bathhouses, showers, and restrooms) activities.[41,43,94] Regardless of the source of fecal contamination, there is considerable evidence that fecal indicator bacteria and viruses, including *E. coli*, *Enterococcus*, *Bacteroides*, *Clostridium perfringens*, and F^+-specific and somatic coliphage (used to

indicate the potential presence of fecal pathogens), have been found to be present orders of magnitude higher in beach sand compared to nearby bathing waters.[40,41,43]

The results of these numerous exposure assessment studies suggest that beach sand contact activities may be associated with health effects; however, a consistent relationship with health effects has not been demonstrated across previous studies.[32,33,41,124] Whereas the health effects,[59] economic burden,[59] and severity of illness [93,143-145] associated with bathing in fresh and marine recreational waters has been well-studied (demonstrating a positive relationship between swimming in fresh and marine recreational water and enteric illness), little is known about the relationship between specific sand contact activities and health effects. Although numerous exposure assessment studies have provided useful information about beach sand quality, very few studies have examined whether specific beach sand contact activities are associated with an increased risk of illness among beach-goers.[32,33,41,124] The relationship between beachgoer reports of specific beach sand contact activities and health effects therefore remains largely unresolved.

The National Epidemiological and Environmental Assessment of Recreational (NEEAR) Water Study is a large national survey of beachgoers sponsored by the EPA and the Centers for Disease Control and Prevention (CDC).[9,146] Using data gathered from beachgoers participating in the 2003-2005 and 2007 rounds of the NEEAR Water Study, we examined the relationship between specific beach sand contact activities (digging in the sand or building sand castles; having one's body buried in the sand) and the risk of enteric [gastrointestinal (GI) illness and diarrhea] and non-enteric illnesses [upper respiratory illness (URI), skin rash, eye ailments, earache, and infected cuts]. To the best

of our knowledge this is the most comprehensive investigation of the association between specific beach sand contact activities and risk of illness.

2. Methods

    a. Study Design / Participant Sampling

The NEEAR Water Study is a prospective cohort design conducted of visitors to freshwater Great Lake beaches on Lake Michigan and Lake Erie during the summers of 2003 and 2004 and also visitors to marine water beaches on the Gulf of Mexico and the Atlantic Coast during the summers of 2005 and 2007. The NEEAR Water Study was designed to evaluate microbial water quality at U.S. beaches and swimming-associated illness. Examining relationships between beach sand contact and illness was originally not a goal of the NEEAR water studies. The data collection methods of the NEEAR water studies have been described previously.[15,146] In brief, we attempted to enroll all beach-goers between 11:00 AM and 5:00 PM during summer weekends and holidays. Unaccompanied minors (below 18 years) or those who could not speak English or Spanish were ineligible.[9,146] At the time of enrollment, we interviewed volunteers to collect baseline information on demographic characteristics and exposures and illnesses that occurred during the previous 3 days. We interviewed volunteers again as they were leaving the beach to ascertain information about their degree of contact with beach sand, swimming behaviors, and other beach activities. Ten to 12 days later, one of the adults in the household was interviewed by telephone about health symptoms experienced by participating household members. We used a standard questionnaire from year to year to collect all demographic, exposure activity, covariate, and illness information from study participants at beaches. Because of the acute nature and short duration of illnesses and

infections considered during this study, participants could re-enroll in the study 28 days after their previous enrollment.

b. Beach Descriptions

Seven beaches with nearby sewage treatment plant discharges were chosen for the NEEAR Water Studies.[9,146] Human-derived pollution sources generally cause the most health concern at recreational beaches.[162] In 2003, NEEAR water studies were conducted at West Beach (on Lake Michigan in Indiana Dunes National Lakeshore in Porter, Indiana) and Huntington Beach (on Lake Erie in Bay Village, Ohio). In 2004, 2 Lake Michigan beaches were studied: Silver Beach, near St. Joseph, Michigan, and Washington Park Beach in Michigan City, Indiana. In 2005, a marine water beach was studied: Edgewater Beach, on the Gulf of Mexico near Biloxi, Mississippi. In 2007, we studied 2 additional marine water beaches: Fairhope Beach, on Mobile Bay in Fairhope, Alabama, and Goddard Beach, on Greenwich Bay near Warwick, Rhode Island.

c. Definition of Sand Contact

We ascertained the nature of participants' contact with beach sand through a structured interview as beach-goers were leaving the beach. The interview included questions about sand exposure, important potential risk factors for sand exposure, important potential risk factors for the illnesses studied, and other activities during participants' time spent at the beach. We asked all participants to give yes or no answers to questions, but participants could refuse to answer any question or report that they didn't know the answer. We considered two types of sand exposure: (1) digging in the sand; and (2) having one's body buried in the sand. Participants who "dug in the sand"

were defined as those who reported that they dug in the sand or built sand castles during the time at the beach the day of the interview. Having one's body buried in the sand was defined as those who reported that they had their body buried in the sand during the time spent at the beach on the day of the interview. We suspected that body buried in the sand could be a more intense exposure to sand. During the 2007 NEEAR water studies, we also collected more specifically defined information on sand exposures including whether participants got sand in their mouth, whether participants ate or drank after playing in the sand, and whether participants washed their hands before eating or drinking after playing in sand.

d. Exposure Period

The exposure period encompasses recreational activities performed during the day of enrollment while participants were at the beach. This was the period between the baseline enrollment interview and the beach exit interview. The enrollment interview was conducted as participants arrived at the beach and the exit interview was conducted after completion of enrollment-day recreational activities as participants were leaving the beach. During the beach enrollment interview participants were asked about the exposures and activities that occurred three days prior to enrollment and during exit interviews participants were asked about the exposures and activities that occurred on the day of enrollment, respectively. Exposures that occurred 3 days before the day of enrollment included activities such as swimming at a pool, swimming at a beach, and exposures that were considered potential confounders of the exposure of interest such as eating raw meat, runny eggs, or shellfish. Some of these exposure questions were

repeated during the telephone follow-up interview conducted 10-12 days after participants departed the beach.

e. Health Assessments

NEEAR water study participants were interviewed at baseline and asked to report physical health symptoms and illness experienced during the 3 days prior to enrollment. We scheduled a follow-up telephone interview as participants departed from the beach on the day of enrollment. Participants who agreed were contacted by telephone 10-12 days following the day of beach exposure and asked to report if they had experienced any of the following physical symptoms or illnesses since their beach-exit interview the day of enrollment:

1. "Gastrointestinal illness" (GI illness) was defined as any of the following: diarrhea (three or more loose stools in a 24-hour period); vomiting; nausea and stomach ache; nausea or stomach ache, and interference with regular activities (missed time from work or school, or missed regular activities as a result of the illness).
2. "Upper respiratory illness" (URI) was defined as any 2 of the following: sore throat, cough, runny nose, cold, or fever.
3. "Rash" was defined as a rash or itchy skin.
4. "Eye ailments" were defined as either eye infection or watery eye.
5. "Earache" was defined as earache, ear infection, or runny ears.
6. "Infected cut" was defined as a cut or wound that became infected

During the telephone follow-up interview, participants answered a series of questions about the occurrence of physical symptoms and illnesses. Ascertainment of

physical symptoms and illness at baseline (3 days prior to enrollment) and again after 10 to 12 days of follow-up allowed us to differentiate between pre-existing or prevalent health outcomes and incident health outcomes. During the telephone follow-up interview we also asked participants about other potential risk factors since the enrollment-day interview. This included information such as the number of times participants went to the same beach, went swimming at another beach (swimming defined as any water contact), went swimming in a pool, or ate raw or under-cooked foods (e.g., red meat, fish, shellfish, eggs). Participants with prevalent illness were excluded from the analysis for that outcome and were eligible to be included in analyses of other outcomes. We also examined a definition of GI illness as diarrhea alone (three or more loose stools in a 24-hour period).

f. Statistical Analysis

A preliminary review of demographic, sand exposure, health outcome, and covariate data was performed to check for outliers and implausible data points. Errors in data were evaluated and, if deemed actual errors by comparisons with responses of other participants and based upon substantive reasoning, were deleted. Analysis data sets were also cleaned and evaluated for missing values. Only those with complete data on important covariates were kept. This involved creation of data sets for each outcome that excluded missing data values for that outcome, sand exposure variables, and critical covariates (age, sex, race/ethnicity, swimming status, and beach).

We examined the frequency of each beach sand contact activity – digging in the sand or building sand castles; having one's body buried in the sand – first stratified by beach and then across all beaches combined. We used log-linear binomial regression

models to estimate the crude and adjusted incidence proportion ratio (IPR) and 95% confidence intervals (CI) for each outcome and its association with each beach sand contact activity. For models comparing those who dug in the sand to those who did not dig in the sand, the aIPR can be interpreted as the risk of illness among those who dug in the sand divided by the risk of illness among those who did not dig in the sand.

We assumed the household to be the unit of independence in the data. There are no individual identifiers in the data (e.g., name, Social Security number, address). To identify potential re-enrollees we matched observations on age (birth date), sex, race/ethnicity, ZIP code, beach and several chronic conditions (Crohn's disease, irritable bowel syndrome, asthma, emphysema / COPD, and chronic skin problems such as psoriasis or eczema). Observations that matched on all of these factors were considered potential re-enrollees. To account for the nonindependence of re-enrollment, the unique household ID of the re-enrollment study entry date was re-assigned to the unique household ID of the first study entry date. Robust estimates of variance were used to account for the nonindependence of observations within household.[163,164]

We evaluated relationships between the exposure (beach sand contact activities), health outcomes, and covariates that were important potential confounders. We considered covariates strongly associated with beach sand contact and illness or those regarded by investigators to be potential confounding factors for inclusion in regression models. These factors included age, sex, race/ethnicity, swimming (defined as any contact swimming), beach, contact with animals, contact with other persons with diarrhea, number of other visits to the beach, any other chronic illnesses (GI, skin, asthma) and eating any food or drink while at the beach. An indicator variable was also

created for a festival that took place at Silver Beach, drawing 17,000 visitors to an area adjacent to the beach. For URI, rash, infected cuts, and eye outcomes, use of insect repellent and sun block were also considered. For each analysis, the set of covariates was reduced through a change-in-estimate procedure.[142] The $aIPR_{full}$ and $aIPR_{reduced}$ were compared using the formula: $\ln|RR_{full}/RR_{reduced}| * 100$. A criterion of a 5% change was used. For the pooled analysis, the covariates in models included age, sex, race/ethnicity, any contact swimming, and beach. For the sub-group analyses, the covariates in models included age, sex, and beach, and selection through change-in-estimate procedure from the following: race/ethnicity, any contact swimming, contact with animals, contact with other persons with diarrhea, eating food while at the beach, eating raw or undercooked meat since the time of the interview, eating raw or undercooked eggs since the time of the interview, number of other visits to the beach, and any other chronic illnesses (GI, skin, asthma). For upper respiratory illness and skin outcomes, insect repellent and sum block use were also considered. The selection procedure generally reduced the number of covariates to 7 or fewer.

Because age was strongly associated with sand exposure and the illnesses considered, we examined the functional form of the distribution of each of the illnesses with respect to age. We plotted incident cases of illness using five-year age intervals (except age groups for the very young: 0 - < 1 yr, 1 – < 2 yr, 2 – 4 yr, and the elderly: 65 – 103 yr). After examining the functional form of the distribution of each of the health outcomes by age, we considered several methods to control for age including: (1) linear age; (2) polynomial age; and (3) age regression splines (linear, quadratic, and restricted quadratic). The goodness of fit of each method of age coding was evaluated by

comparing akaike's information criteria (AIC) values (the age adjustment method that resulted in the lowest AIC value was chosen). Choice of spline age variable knots was informed by examining the inflection points of the plots of the illness incidence proportion distributions by age. We observed an age-sex crossover in the incidence proportion of enteric illnesses (GI illness and diarrhea). To model this observed age-sex crossover more precisely, multiplicative interaction coding between age and sex variables was used in log-linear binomial regression models for the enteric illnesses (GI illness and diarrhea). This process (of examining the functional form of the incidence proportion by age) was used for each of the other outcomes. Non-enteric illnesses showed a linear trend of decline in the age-specific incidence proportions and no age-sex crossover. For the non-enteric illnesses (URI, skin rash, eye ailments, earache, infected cuts) linear age was used in regression models.

We also examined potential effect measure heterogeneity (on the multiplicative scale) of the sand exposure/illness relationship across strata of covariates with biological plausibility as potential effect measure modifiers. We first examined potential heterogeneity across the 7 beaches, then by swimming status. Multiplicative interaction terms were coded for: (1) sand exposure and beach; and (2) sand exposure and swimming status. This allowed slopes to differ across beaches and then across levels of swimming status. Because of sample size limitations we were unable to estimate interaction effects for all stratified estimates. For covariates that were not biologically plausible effect measure modifiers, or were not estimable because of small cell sizes, we reported combined effect measure estimates adjusted for potential confounders.

Because previous analysis demonstrated a high sensitivity of children to swimming-associated gastrointestinal illness, [146] (and because age was observed to be strongly associated with the outcomes), a stratified analysis was conducted for age in the following categories: (1) 0 to 10 years (children); (3) 11-54 years (children and adults); and (3) 55-103 years (older adults). The age groups were selected based on sample size considerations and previous research.[146] All analyses were completed using SAS version 9 © (SAS Institute Inc., Cary, NC, USA) and Stata version 9 © (StataCorp LP, College Station, TX, USA).

3. Results

A total of 27,365 interviews from 13,220 household groups were completed. Of these interviews, 26,339 interviews had complete information on the full set of key covariates: age, sex, race/ethnicity, contact with beach sand, and water contact status (swimming) and were included for further analysis. Relationships between sand exposure and each of the health outcomes were evaluated using interviews with complete exposure, outcome, and covariate information.

Respondents at the 7 beaches differed by age, race/ethnicity, miles traveled to the beach, and proportion of individuals who reported digging in sand. Respondents were 80% white and 56% female, with a median age of 28 years. Those who dug in the sand were younger than those who did not dig in the sand (median age 14 and 35 years, respectively) but were equally likely to report vomiting, other GI symptoms, rash, eye irritation, earache, and a history of chronic respiratory problems or asthma at baseline (Table 1). Fewer individuals who dug in the sand reported a history of chronic GI illness (2% vs. 3%), a history of chronic allergies (18% vs. 20%), and consumption of red or raw

meat prior to or immediately after the beach visit (8% vs. 11%). More individuals who dug in the sand reported an infected cut or wound at baseline (8% vs. 5%), contact with animals 48 h prior to or immediately after the beach visit (77% vs. 72%), and swimming, defined as any contact with the water (82% vs. 52%). Those who had their body buried in the sand were also younger than those who did not have their body buried in the sand (median age 10 and 30 years, respectively), but were equally likely to report vomiting, other GI symptoms, sore throat, eye irritation, and earache at baseline (Table 1). Fewer individuals who had their body buried in the sand reported a history of allergies (17% vs. 19%), a history of chronic GI illness (1% vs. 3%), and consumption of red or raw meat 48 h prior to or immediately after the beach visit (7% vs. 10%). However, individuals who had their body buried in the sand were more likely to have reported an infected cut at baseline (8% vs. 6%), swimming the day of the beach interview (89% vs. 62%), and have had contact with animals 48 h prior to or immediately after the beach visit (77% vs. 74%).

There were more female beach-goers than male in both sand-contact groups (digging in sand and body buried in the sand), with the largest discrepancy among those who did not dig in the sand (58% vs. 42%) and the smallest among those who reported having their body buried in the sand (51% vs. 49%). Most participants in contact with sand were white. The percentages of other races were similar across sand-contact groups, except Whites who were less likely to have their body buried in the sand (77% vs. 81%) and Hispanic/Latino participants who were more likely to report being buried in the sand (14% vs. 10%). More swimmers than non-swimmers reported digging in the sand (82% vs. 18%) and being buried in the sand (89% vs. 11%). Participants at Washington Park

Beach reported digging in the sand (47%) and buried in the sand (16%) most frequently followed by participants at West Beach who were slightly less likely to have reported digging in the sand (46%) being buried in the sand (13%).

a. Relationship Between Sand Contact Activities and Illness

The incidence of GI illness was 7.3% (1863 of 25548 without baseline illness) during the 10 to 12 day follow-up period. GI illness incidence was highest among children 10 years of age and younger (9%) and lowest among those aged 55 and older (5%). The adjusted risk of GI illness among those who dug in the sand was 1.14 times the risk of GI illness among those who did not dig in the sand (95% CI = 1.02–1.26; Table 2). The incidence of diarrhea was 4.9% (1258 of 24471) during the 10 to 12 day follow-up period. Diarrhea incidence was highest among children 10 years of age and younger (6%) and lowest among those aged 55 and older (5%). The adjusted risk of diarrhea among those who dug in the sand was 1.20 times the risk of diarrhea among those who did not dig in the sand (95% CI = 1.05–1.36; Table 2).

Approximately 6% of respondents reported URI and incidence was highest among children 10 years of age and younger (8%) and lowest among adults 55 years of age and older (3%). The crude incidence of URI was higher among those who dug in the sand compared to those who did not dig in the sand, but after adjustment there was little difference in risk (aIPR = 1.06; 95% CI = 0.93–1.20; Table 2). Age was a strong confounder because younger respondents were more likely both to dig in the sand and report URI. The remaining non-enteric illnesses did not show a strong or consistent positive association with digging in the sand.

Generally, the association between a more intense sand exposure, being buried in the sand, and enteric illness was stronger than the association between digging in the sand and enteric illness. The adjusted risk of GI illness among those who had their body buried in the sand was 1.22 times the adjusted risk of GI illness among those who did not have their body buried in the sand (95% CI = 1.04–1.42; Table 2). The adjusted risk of diarrhea among those who had their body buried in the sand was 1.23 times the adjusted risk of diarrhea among those who did not have their body buried in the sand (95% CI = 1.01–1.51; Table 2). For the non-enteric illnesses studied no consistent increase in risk was observed among those buried in the sand compared to those not buried in the sand (Table 2).

Stronger and more consistent positive associations between sand contact activities and enteric illnesses were observed among children 10 years of age and younger. An elevated risk of GI illness was observed among children who dug in the sand (1.29; 95% CI = 0.97–1.71; Table 3). There was no evidence of risk among adults 55 years of age and older (0.91; 95% CI = 0.54–1.55; Table 3). Diarrhea risk was highest among children 10 years of age and younger digging in the sand (1.46; 95% CI = 1.01–2.12; Table 3) and lowest among those 55 years of age and older (1.01; 95% CI = 0.57–1.81; Table 3). The risk of GI illness was elevated among children who had their body buried in the sand (1.30; 95% CI = 1.04–1.62; Table 3). However, the GI illness risk was also elevated among those 11 to 54 years of age (1.24; 95% CI = 1.01–1.53; Table 3) and somewhat among adults 55 years of age and older (1.27; 95% CI = 0.41–3.94 ; Table 3). Due to a low illness incidence and low exposure prevalence, the effect estimate among adults 55 years of age and older was imprecise (Table 3). Diarrhea risk was highest

among children 10 years of age and younger buried in the sand (1.30; 95% CI = 0.98–1.71; Table 3) and lowest among those 55 years of age and older (1.08; 95% CI = 0.27–4.36; Table 3). The effect estimate for those 55 years of age and older was imprecise (Table 3) due to the low incidence of diarrhea (4%) and low prevalence of exposure (2%) in this sub-group. The non-enteric illnesses did not show a strong or consistent positive association with either of the sand contact activities among the age sub-groups.

The risk of illness following sand exposure showed considerable variation across the beaches studied. Among those digging in the sand, effect estimates ranged from 0.99 to 1.89 (Table 4). The strongest associations were among those who dug in the sand at Fairhope Beach with GI illness (1.50; 1.03–2.20; Table 4) and diarrhea (1.89; 1.25–2.84; Table 4). The weakest associations were among those who dug in the sand at Huntington Beach with GI illness (0.99; 0.77-1.27) and with diarrhea (1.01; 0.76-1.36). There was also variation by type of beach (marine vs. freshwater). Risks of enteric illnesses were higher at marine beaches than at freshwater beaches. At the marine beaches, the adjusted risk of diarrhea among those who dug in the sand was 1.46 times the adjusted risk of GI illness among those who did not dig in the sand (1.09–1.95; Table 4). This association was also somewhat elevated for GI illness at marine beaches (1.26; 0.99–1.60; Table 4). Similar variability was observed among those with their body buried in the sand (Table 4). There was little observed evidence of consistent swimming-specific variation in the effect of sand contact on risk of illness. Overall, the results of analyses of associations between beach sand contact activities and non-enteric illnesses demonstrated a weak or small strength of effect.

4. Discussion

Results of our study suggest that, among beach-goers participating in a large prospective cohort study, reported contact with beach sand (defined as either digging in the sand or having one's body buried in the sand) was associated with an elevated risk of enteric illnesses (GI illness and diarrhea) in the 10-12 days following exposure. Being buried in the sand appeared to be a more intense sand exposure as evidenced by higher point estimates for enteric illness compared to those for the digging in the sand exposure. We also observed a higher proportion of people who got sand in their mouth among those buried in the sand compared to those who dug in the sand. Data from more specifically defined sand exposure questions collected during the 2007 NEEAR water study revealed that participants buried in the sand reported getting sand in their mouth nearly twice as frequently as those who dug in the sand (39.8% vs. 20.4%).

Although an elevated risk of enteric illness from contact with beach sand has been hypothesized, ours is one of the first studies to demonstrate an association with specific sand contact activities. One previous study demonstrated that time spent in the wet sand was associated with a dose-dependent increase in GI illness (per 10 minute increase in time spent in contact with wet sand an adjusted OR = 1.008; 95% CI = 1.001-1.015).[41] However, the authors cautioned that this finding needed to be validated by future epidemiologic studies.[41] The variability observed across beaches, however, indicates that risks may be site-specific, and may depend on characteristics of each individual beach.

We examined variation in the relationship between sand contact and illness in sub-groups of age. The results provided some evidence that children ≤10 years of age may be at higher risk of GI illness and diarrhea following digging in the sand. In general, the enteric illness risk of digging in the sand was lower in the two older age sub-groups

(11-54 years and 55+ years) than children (0-10 years). However, for the body buried in the sand exposure (considered a more intense sand exposure) elevated enteric illness risks were observed among children (0-10 years of age) and also among the other age groups (11-54; 55+ years of age). This suggestion of increased enteric illness risk among children (0-10 years) could be due to a susceptibility to illness following exposure or a prolonged or more intense exposure to sand. In our study, children (0-10 years) had a much higher frequency of exposure to sand (79% reported digging in sand) as well as a higher frequency of getting sand in their mouth (18.9%) compared to older children and adults (11-54 years) (4.1%) and older adults (55+ years) (1.8%). A recent study of soil ingestion among parents and their children by Davis et al., showed that soil ingestion was highest among children.[165] We observed some evidence of increased diarrhea risk among older adults (55+ years) who had their body buried in the sand. For example, even though there was some evidence of increased GI illness risk for the body buried in the sand exposure among children (0-10 years), children and adults (11-54 years), and older adults (55+ years), GI illness risk was most elevated among older adults (55+ years). Our ability to make valid conclusions about enteric illness risks among those 55 years and older buried in the sand was limited because this group was exposed infrequently and also reported the lowest incidence of enteric illness. The enteric illness effect estimates for those aged 55 years and older were therefore imprecise and must be interpreted with caution.

We observed beach-specific variation in the enteric illness risk following beach sand contact. This could have been due to a number of site-specific differences at the beaches studied, including factors such as sand composition (e.g., clay, silt, loam),

particle size, moisture and organic content, nutrient availability, osmotic pressure, tidal phenomena, wave action, currents, and algae/seaweed density that may impact the sand.[45,68] Human factors such as bather density and use of sand grooming practices (such as daily tilling) may also impact beach sand quality.[17,121,138,166] The observed variation in enteric illness risks across beaches could also be explained by different levels of fecal pollution influencing recreational waters and subsequently sand from sources including: publicly owned treatment works (POTW) sewage discharges, non-point source run-off (e.g., domestic and wild animals, urban stormwater), and bather density. Each of these factors may impact beach sand quality.

It is possible that variation between marine and freshwater risks could be explained by differential survival of enteric pathogens in beach sand impacted by marine vs. freshwater. Different sand characteristics (at marine vs. freshwater beaches) mentioned above including particle size, clay content, salinity, etc. may also play a role. It has been observed that salinity increase is inversely correlated with the survival of bacterial indicators of the presence of feces in recreational waters, however, data to explore this relationship and other sand characteristics relationships in sand were not available during the present study.[167] It is also possible that there were differences in levels of fecal pollution at the marine vs. freshwater beaches studied. The observed beach-specific variation in risk of enteric illness and the stronger association between sand contact and enteric illness at marine beaches warrant further investigation. There was little evidence of consistent swimming-specific variation in the effect of sand contact activities on risk of illness. To attempt to disentangle the effect of digging in the sand from swimming, we examined associations with enteric illness among nonswimmers who

dug in the sand. There was a similar effect among nonswimmers who dug in the sand for GI illness (aIPR = 1.26; 95% CI = 1.02-1.55) and diarrhea (aIPR = 1.26; 95% CI = 0.97-1.62) compared to our results among all participants who dug in the sand.

We observed beach-specific variation in frequency of reports of sand contact that may reflect the potential influence of site-specific characteristics on exposure, including sand type and aesthetic and physical characteristics of sand (e.g., rocky sand vs. fine/grainy sand, dark color vs. light color). Data from more specific sand exposure questions collected during 2007 at 2 marine beaches revealed that fewer participants reported any sand contact (digging in sand or being buried in sand) at Goddard Beach (25.6%) where sand was rocky and dark-colored compared to Fairhope Beach (61.3%) where sand was more fine, grainy, and light-colored. Data from more specific sand exposure questions of getting sand in one's mouth, eating or drinking, and handwashing after sand contact at both beaches revealed that 20.3% reported getting sand in their mouth, 48.8% reported eating or drinking anywhere after sand contact, and over half (59.1%) reported not washing their hands before eating or drinking after sand contact.

Some of the illnesses studied were nonspecific (e.g., GI illness, eye irritation) and may have been affected by recall bias. We expected that recall would have been nondifferential with respect to sand exposure status. Therefore there would have been limited influence by recall bias (although there could have been a loss of precision due to potential under-recall/reporting of illness). The illness categories were broad endpoints, the association between contact with sand and enteric illnesses was robust to varying definitions of sand contact (digging in the sand and being buried in the sand) and definitions of enteric illness (GI illness and diarrhea). The association was not robust

across all 7 beaches as we observed variation in the beach-stratified point estimates, suggesting potential site-specific factors may influence the risk of illness following sand contact. Few studies have evaluated associations between beach sand exposure and symptoms of illness.[32,41,124] Previously, observed associations with symptoms of illness have been inconsistent and only one study demonstrated an association between time spent in contact with wet sand and GI illness.[41] Our results are consistent with this study's finding of an association between sand contact and GI illness. Relationships between beach sand contact and nongrastrointestinal (nonenteric) health conditions appeared to be less consistent. Similar to previous studies, we found no consistent relationship between nonenteric illnesses and contact with beach sand.[32,41,124]

We do not know if the relationships we observed between contact with beach sand and symptoms of illness can be extended to relationships with concentrations of fecal indicator organisms in beach sand, or to beaches affected by different sources of fecal contamination in water (non-point sources of pollution). During the NEEAR water studies (2003-2005 and 2007), all beach sites had evidence of fecal contamination (by measurement of fecal indicator bacteria and F^+-specific coliphage in water). In 2007 sand samples were collected at 2 beaches. A detailed analysis of concentrations of fecal indicators will be presented in future research. The findings of the present study suggest a need for future epidemiologic investigation of relationships with quantitative measures of beach sand quality (i.e., fecal indicator organism concentrations in beach sand) along with prospective ascertainment of beach sand exposure activities and symptoms of illness. Previous studies of swimming exposure have demonstrated stronger associations with symptoms of illness compared to the associations reported in our present study of

beach sand exposure and illness.[9,146] However, our results suggest that contact with beach sand was associated with enteric illness risk at certain beaches. Future studies should focus on better defining this risk and understanding factors that contribute to fecal contamination of sand.

5. Conclusions

Contact with beach sand appears to increase the risk of enteric illness. Sand contact may be particularly important as a source of risk for young children because they may not swim, but play in the sand. There may be potential to reduce the observed increased risk of enteric illness by reducing levels of fecal pollution from municipal sewage discharges and non-point sources that may impact sand as well as recreational water.

Table 1. Characteristics of Those Who Did Not Dig in the Sand, Those Who Dug in the Sand, Those Who Did Not Have Their Body Buried in the Sand, and Those Who Did Have Their Body Buried in the Sand.

	Digging in the Sand		Body Buried in the Sand	
	No (n = 15685) No. (%)[a]	Yes (n = 10654) No. (%)[a]	No (n = 23905) No. (%)[a]	Yes (n = 2436) No. (%)[a]
Age (yrs)				
0-4	503 (3)	1651 (15)	1732 (7)	423 (17)
5-10	646 (4)	2703 (25)	2454 (10)	894 (37)
11-19	2154 (14)	1724 (16)	3418 (14)	462 (19)
20-54	10525 (67)	4236 (40)	14144 (59)	618 (25)
55+	1857 (12)	340 (3)	2157 (9)	39 (2)
Sex				
Male	6593 (42)	4948 (46)	10338 (43)	1201 (49)
Female	9092 (58)	5706 (54)	13559 (57)	1234 (51)
Race/Ethnicity				
White	12747 (81)	8498 (80)	19364 (81)	1874 (77)
Black	981 (6)	642 (6)	1486 (6)	137 (6)
Asian	229 (1)	59 (1)	368 (2)	20 (<1)
American Indian	49 (<1)	26 (<1)	66 (<1)	9 (<1)
Hispanic/Latino	1584 (10)	1173 (11)	2405 (10)	352 (14)
Multiethnic/other	95 (<1)	156 (1)	208 (<1)	43 (2)
Miles traveled to the beach				
0-5 miles	3856 (25)	2442 (23)	5859 (25)	435 (18)
6-20 miles	4297 (28)	2432 (23)	6162 (26)	566 (24)
21-50 miles	3892 (25)	2810 (27)	5983 (25)	717 (30)
50 miles or greater	3472 (22)	2845 (27)	5633 (24)	684 (28)
Conditions in the 3 d prior to the beach visit				
Vomiting	166 (1)	106 (1)	245 (1)	27 (1)
Other GI symptoms	361 (2)	226 (2)	540 (2)	47 (2)
Sore throat	822 (5)	645 (6)	1323 (6)	144 (6)
Rash	347 (2)	264 (2)	538 (2)	73 (3)
Sunburn	839 (5)	438 (4)	1182 (5)	94 (4)
Infected cut	849 (5)	837 (8)	1488 (6)	198 (8)
Eye irritation	79 (<1)	53 (<1)	117 (<1)	15 (<1)
Earache	195 (1)	157 (1)	317 (1)	35 (1)
History of chronic respiratory problems or asthma	1027 (7)	718 (7)	1588 (7)	156 (6)
History of allergies	3060 (20)	1911 (18)	4565 (19)	404 (17)
History of chronic GI illness	503 (3)	184 (2)	658 (3)	29 (1)
Any history of chronic GI illness, asthma, or allergies	3061 (20)	1913 (18)	4567 (19)	405 (17)
Water contact status				
No water contact	7510 (48)	1945 (18)	9185 (38)	267 (11)
Water contact	8175 (52)	8709 (82)	14712 (62)	2168 (89)
Contact with animals 48 h prior to or after beach visit, or between beach visit and phone interview	11374 (72)	8241 (77)	17745 (74)	1864 (77)
Consumption of red, raw or undercooked meat 48 h prior to beach visit or between beach visit and phone interview	1754 (11)	878 (8)	2453 (10)	178 (7)
Beach				
Goddard Beach	2270 (14)	574 (5)	2743 (11)	101 (4)
Fairhope Beach	1243 (8)	752 (7)	1885 (8)	110 (5)
Edgewater Beach	826 (5)	483 (5)	1208 (5)	101 (4)
Washington Park Beach	2174 (14)	1964 (18)	3487 (15)	649 (27)
Silver Beach	5726 (37)	4678 (44)	9440 (40)	961 (40)
Huntington Beach	1913 (12)	903 (8)	2682 (11)	134 (6)
West Beach	1533 (10)	1300 (12)	2452 (10)	379 (16)

[a]Excludes those with missing information on age, sex, race/ethnicity, water contact status, and beach.

TABLE 2. Illness Incidence According to Sand Exposure and Adjusted Incidence Proportion Ratios (aIPR) Comparing Those With Sand Exposure to Those Without Sand Exposure

	Incidence			
	Digging in the Sand			
	No	Yes	No.	
Illness	**No. (%)**	**No. (%)**	**Observations**	**aIPR (95% CI)**
GI	999 (7)	864 (8)	25548	1.14 (1.02-1.26)
Diarrhea	671 (4)	587 (6)	25729	1.20 (1.05-1.36)
Respiratory illness	711 (5)	667 (7)	24869	1.06 (0.93-1.20)
Rash	400 (3)	319 (3)	25716	1.02 (0.85-1.21)
Eye ailments	506 (3)	265 (3)	26204	0.86 (0.73-1.03)
Earache	210 (1)	181 (2)	25981	1.05 (0.84-1.32)
Infected cuts	72 (<1)	40 (<1)	26328	0.70 (0.45-1.08)
	Incidence			
	Body Buried in Sand			
	No	**Yes**	**No.**	
Illness	**No. (%)**	**No. (%)**	**Observations**	**aIPR (95% CI)**
GI	1639 (7)	224 (9)	25541	1.22 (1.04-1.42)
Diarrhea	1112 (5)	146 (6)	25722	1.23 (1.01-1.51)
Respiratory illness	1238 (5)	140 (6)	24862	0.86 (0.70-1.05)
Rash	644 (3)	75 (3)	25709	0.98 (0.76-1.26)
Eye ailments	709 (3)	62 (3)	26197	1.00 (0.75-1.34)
Earache	361 (2)	30 (1)	25974	0.67 (0.45-0.99)
Infected cuts	100 (<1)	12 (<1)	26321	1.11 (0.58-2.12)

Numbers are those reporting new symptoms, among those without baseline symptoms. For GI reporting illness, subjects vomiting or other GI symptoms in the past 3 d shown in Table 1 were excluded. aIPR estimated from log-risk binomial regression models adjusted for age, sex, race/ethnicity, beach, and swimming.

Table 3. Adjusted Incidence Proportion Ratios (aIPR) for Illness Comparing Those With Sand Exposure to Those Without Sand Exposure, by Age Group

	Age Group (Years)					
	0-10		11-54		>55	
	Incidence No. (%)	aIPR (95% CI)	Incidence No. (%)	aIPR (95% CI)	Incidence No. (%)	aIPR (95% CI)
			Digging in the Sand			
GI Illness	446 (9)	1.23 (0.93-1.62)	1268 (7)	1.12 (1.00-1.26)	107 (5)	0.90 (0.54-1.51)
Diarrhea	291 (6)	1.45 (1.01-2.09)	848 (5)	1.14 (0.98-1.32)	82 (4)	1.03 (0.58-1.81)
URI	413 (8)	1.15 (0.86-1.54)	875 (5)	1.09 (0.94-1.25)	51 (3)	1.13 (0.54-2.38)
Rash	178 (3)	0.89 (0.61-1.30)	486 (3)	1.10 (0.90-1.33)	39 (2)	0.47 (0.17-1.35)
Eye	110 (2)	0.93 (0.54-1.59)	558 (3)	0.84 (0.70-1.02)	78 (4)	1.42 (0.77-2.62)
Earache	96 (2)	0.80 (0.47-1.35)	256 (1)	1.20 (0.93-1.55)	18 (<1)	0.87 (0.17-4.41)
Infected Cuts	22 (<1)	0.65 (0.23-1.84)	80 (<1)	0.75 (0.46-1.21)	5 (<1)	--
			Body Buried in the Sand			
GI Illness	446 (9)	1.21 (0.97-1.51)	1268 (7)	1.24 (1.00-1.53)	107 (5)	1.43 (0.44-4.68)
Diarrhea	291 (6)	1.23 (0.94-1.63)	848 (5)	1.19 (0.91-1.55)	82 (4)	1.24 (0.29-5.30)
URI	413 (8)	0.85 (0.66-1.10)	875 (5)	1.02 (0.76-1.36)	51 (3)	--
Rash	178 (3)	0.83 (0.57-1.21)	486 (3)	1.26 (0.90-1.77)	39 (2)	1.49 (0.22-10.24)
Eye	110 (2)	1.40 (0.90-2.17)	558 (3)	0.92 (0.61-1.37)	78 (4)	--
Earache	96 (2)	0.61 (0.34-1.08)	256 (1)	0.80 (0.46-1.40)	18 (<1)	--
Infected Cuts	22 (<1)	1.07 (0.39-2.91)	80 (<1)	1.35 (0.56-3.24)	5 (<1)	--

Numbers are those reporting new symptoms, among those without baseline symptoms. aIPR estimated from log-risk binomial regression models. Covariates in models included age, sex, and beach, and selection through change in estimate procedure from the following: race/ethnicity, any contact swimming, contact with animals, contact with other persons with diarrhea, eating food while at the beach, eating raw or undercooked meat since the time of the beach interview, eating raw or undercooked eggs since the time of the beach interview, number of other visits to the beach, and any other chronic illnesses (GI, skin, asthma). For upper respiratory illness and skin outcomes, insect repellent and sun block use were also considered.

Table 4. Adjusted Incidence Proportion Ratios (aIPR) for Illness Comparing Those With Sand Exposure to Those Without Sand Exposure, by Beach, and by Marine versus Fresh Water Beaches

	Illness			
	GI Illness		Diarrhea	
	Incidence No. (%)	aIPR (95% CI)	Incidence No. (%)	aIPR (95% CI)
	Digging in the Sand			
Beach				
Goddard Beach	130 (5)	1.27 (0.79-2.05)	78 (3)	1.38 (0.73-2.59)
Fairhope Beach	155 (8)	1.50 (1.03-2.20)	113 (6)	1.89 (1.25-2.84)
Edgewater Beach	118 (9)	1.05 (0.69-1.62)	74 (6)	1.12 (0.65-1.93)
Washington Park Beach	287 (8)	1.32 (1.02-1.71)	25 (5)	1.25 (0.92-1.71)
Silver Beach	632 (6)	1.09 (0.90-1.31)	390 (4)	1.20 (0.94-1.54)
Huntington Beach	270 (10)	0.99 (0.77-1.27)	206 (8)	1.01 (0.76-1.36)
West Beach	229 (8)	1.11 (0.83-1.49)	176 (6)	1.03 (0.74-1.43)
Marine Water Beaches				
Goddard, Fairhope, and Edgewater Beaches	403 (7)	1.26 (0.99-1.60)	265 (5)	1.46 (1.09-1.95)
Fresh Water Beaches				
Washington Park, Silver, Huntington, and West Beaches	1418 (7)	1.11 (0.99-1.24)	970 (5)	1.14 (0.99-1.32)
	Body Buried in the Sand			
Beach				
Goddard Beach	130 (5)	1.73 (0.76-3.94)	78 (3)	1.95 (0.61-6.28)
Fairhope Beach	155 (8)	1.25 (0.55-2.81)	113 (6)	1.63 (0.65-4.14)
Edgewater Beach	118 (9)	1.55 (0.82-2.95)	74 (6)	1.79 (0.75-4.27)
Washington Park Beach	287 (8)	0.94 (0.65-1.35)	25 (5)	0.91 (0.57-1.46)
Silver Beach	632 (6)	1.28 (1.01-1.64)	390 (4)	1.18 (0.84-1.65)
Huntington Beach	270 (10)	0.91 (0.49-1.70)	206 (8)	0.85 (0.40-1.79)
West Beach	229 (8)	1.77 (1.25-2.53)	176 (6)	1.89 (1.26-2.83)
Marine Water Beaches				
Goddard, Fairhope, and Edgewater Beaches	403 (7)	1.36 (0.90-2.05)	265 (5)	1.52 (0.89-2.58)
Fresh Water Beaches				
Washington Park, Silver, Huntington, and West Beaches	1418 (7)	1.22 (1.03-1.45)	970 (5)	1.21 (0.97-1.51)

Numbers are those reporting new symptoms, among those without baseline symptoms. aIPR estimated from log-risk binomial regression models. Covariates in models included age, sex, and beach, and selection through change in estimate procedure from the following: race/ethnicity, any contact swimming, contact with animals, contact with other persons with diarrhea, eating food while at the beach, eating raw or undercooked meat since the time of the interview, eating raw or undercooked eggs since the time of the interview, number of other visits to the beach, and any other chronic illnesses (GI, skin, asthma). For upper respiratory illness and skin outcomes, insect repellent and sun block use were also considered.

B. Association between concentrations of fecal indicators in beach sand and risk of GI illness

1. Introduction

The results of recent exposure assessment studies, showing that high levels of *E. coli*, *Enterococcus*, and other indicators of fecal contamination have been isolated from beach sand (often at higher concentrations than in nearby bathing waters), have raised questions about whether beach sand can serve as a vehicle for transmission of pathogens associated with fecal contamination to humans, leading to an increased risk of infection and subsequent symptoms of illness.[40,41,43,45,68,96,110,168] These questions are of increasing concern to beach managers, public health officials, and beach-goers and remain largely unresolved due to inconsistent results from a small number of previous epidemiologic studies.[32,41,124] Because the beach-going public typically spends more time on the beach than in the water and young children often spend most of their time at the water's edge playing in sand, it is important to understand the relationship between fecal indicator concentrations in sand, sand contact activities, and risk of illness. There currently exists limited information on the safety of human exposure to fecal contamination in beach sand. Positive associations between fecal indicator organism concentrations (which are non-pathogenic microorganisms used to indicate the degree of fecal contamination) in recreational water and swimming-associated illness have been well documented.[6,9-11,15,21,33,59,60,146,169] GI illness has most commonly been associated with fecal indicator organisms in recreational water and this informed our focus on GI illness during this study.

In addition to fecal indicator organisms – such as fecal coliforms, *E. coli*, *Enterococcus*, male-specific and somatic coliphage, *Bacteroides*, *B. thetaiotaomicron*, and *Clostridium perfringens* – pathogenic viruses, bacteria, fungi, and parasites have been

isolated from sand at nearshore (sand-water interface) and backshore (dry sand) regions of beaches.[43,52,68,94,96,105,110,170,171] Although human-derived pollution sources generally cause the most health concern at recreational beaches [162] competing theories exist about whether beach sand acts as a net source and/or net sink of fecal contamination. Several studies provide evidence of fecal indicator bacteria (*E. coli* and *Enterococci*) re-growth in sand [63,65,69,110,168,172] while others suggest that point (sewage outfalls) and non-point (wild and domestic animals and humans) sources [63,173] of fecal contamination directly impact beach sand. Survival and dispersion of fecal contamination on beach sand seems to depend on a complicated interplay between numerous factors including latitude (e.g., temperate vs. tropical beach), region of the beach (wet nearshore sand vs. dry backshore sand), the presence of protective conditions providing cover from microbial inactivation and predation (e.g., cladophora mats), tidal phenomena, beach sand grooming practices, the season, and fecal contamination source (e.g., point source sewage outlet vs. non-point source animal vs. human bather inputs).[45] Determining which of these numerous competing factors is the most predominant at a given beach is often challenging.

Notwithstanding uncertainty about the predominant sources of fecal contamination in beach sand, the results of these numerous exposure assessment studies and one recent pilot epidemiological study [41] suggest that increased fecal indicator organism concentrations in beach sand could be related to health effects – particularly GI illness. A consistent relationship between concentrations of fecal indicators and GI illness has not been demonstrated across previous studies, however, Bonilla et al. observed a positive association between time spent in wet sand and GI illness.[32,33,41,124] Very few studies have examined whether increasing concentrations of fecal indicators in beach sand are associated with an

increasing risk of GI illness among beach-goers who engage in specific sand contact activities.[32,33,41,124]

The National Epidemiological and Environmental Assessment of Recreational (NEEAR) water study is a large national survey of beachgoers sponsored by the EPA and the Centers for Disease Control and Prevention (CDC).[9,146] It was designed to investigate relationships between water quality and swimming-associated illness at beaches impacted by municipal sewage discharges. During the 2007 rounds of the NEEAR water study, beach sand samples were collected at two marine beaches (Fairhope Beach, Alabama and Goddard Memorial State Park Beach, Rhode Island and analyzed for the fecal indicator organisms *Enterococcus*, F^+-specific coliphage, *Bacteroides*, and *B. thetaiotaomicron* (which have been reported as indicators of potential fecal contamination).[10,92,135,158,168,174-177] The addition of beach sand sample collection and quality analyses to the NEEAR water study afford an opportunity to examine associations between concentrations of fecal indicator organisms and risk of GI illness following contact with beach sand. It was our aim to explore whether an increase in daily average fecal indicator concentrations (indicating daily average sand quality) led to an increased risk of GI illness: (1) among those engaged in sand contact activities only; and (2) among all participants with those not engaged in contact with sand as the reference category.

2. Methods

    a. Study Design / Participant Sampling

The NEEAR water study is a prospective cohort design conducted in 2007 of visitors to marine beaches on the Gulf of Mexico and the Atlantic Coast during weekends and major holidays of the summer swimming season (from Memorial Day to Labor Day). The data

collection methods of the NEEAR water studies have been described previously.[9,146] In brief, we attempted to enroll all beach-goers between 11:00 AM and 5:00 PM during summer weekends and holidays. We excluded unaccompanied minors (below 18 years) or those who could not speak English or Spanish.[9,146] At the time of enrollment, we interviewed volunteers to collect baseline information on demographic characteristics and exposures and illnesses that occurred during the previous 3 days. We interviewed volunteers again as they were leaving the beach to ascertain information about their degree of contact with beach sand, swimming behaviors, and other beach activities. Ten to 12 days later, one of the adults in the household was interviewed by telephone about health symptoms experienced by participating household members. We used a standard questionnaire to collect all demographic, exposure activity, covariate, and illness information from study participants at beaches. Because of the acute nature and short duration of enteric symptoms and illness considered during this study, repeat enrollment by participants was allowed. Participants were eligible to re-enroll in the study 28 days after their previous enrollment.

b. Beach Descriptions

Beach sites affected by nearby sewage treatment plant discharges were chosen for the NEEAR water studies.[9,146] The 2007 NEEAR water studies were conducted at 2 marine beaches: Fairhope Beach (on Mobile Bay in Fairhope, Alabama) and Goddard Memorial State Park Beach (on Greenwich Bay near Warwick, Rhode Island).

c. Beach Sand Sample Collection and Sample Analysis

Beach sand samples were tested for fecal indicator bacteria *Enterococcus*, *Bacteroides* and *Bacteroides thetaiotaomicron* using a quantitative polymerase chain reaction

cell equivalent (qPCR CCE) method.[136] *B. thetaiotaomicron* is considered a more human-specific indicator of fecal contamination.[75,177,178] Samples were also tested for *Enterococcus* and F^+-specific coliphage using culture-based EPA Method 1600[151] and EPA Method 1601[89], respectively. Beach sand samples were collected at 8:00 AM along with water samples each weekend day of the 2007 NEEAR water studies. Wet sand samples were collected using a 2.25-inch diameter stainless steel soil auger at a distance of 1 meter perpendicular to the lowest point of the water level (when the waves receded to their lowest point on the shoreline) along the same 3 transects where water samples were collected. Transects were located at least 60 m apart to encompass the entire beach area. The soil auger was pushed into the sand at least 8 inches, capped, labeled with a unique alpha-numeric code, sealed in a zip-loc bag, and transported to the laboratory for processing on ice in a cooler maintained at 1 to 4°C. Analyses of *Enterococcus* were performed by local laboratories within 6 hours of collection. Samples were filtered for qPCR analysis within 6 hours of collection. To ensure consistency across the 2 beaches, the filters were frozen and sent on dry ice by overnight express for analysis by EMSL Analytical, Inc. Laboratory (Westmont, NJ). The qPCR method used in this study has been previously described.[9,136,179]

Samples that were analyzed for F^+-specific coliphage were sent on dry ice by overnight express and processed using EPA Method 1601 with some modifications. Fecal indicator organism concentrations are reported as quantitative polymerase chain reaction calibrator cell equivalents (qPCR CCE) for *Enterococcus*, *Bacteroides*, and *Bacteroides thetaiotaomicron*, colony-forming units (CFU) for *Enterococcus* by Method 1600, and plaque-forming units (PFU) for F^+-specific coliphage by Method 1601 per gram of sand. At each sampling time we recorded environmental conditions, including air and water

temperature, cloud cover, UV light, rainfall, wind speed and direction, wave height, beach population density, boats, animals (number and type), and debris.

d. Definition of Sand Contact

We ascertained participants' contact with beach sand through a structured interview as beach-goers were leaving the beach. The interview included questions about sand exposure, important potential risk factors for sand exposure, important potential risk factors for GI illness, and other activities during participants' time spent at the beach. We asked all participants to give yes or no answers to questions, but participants could refuse to answer any question or report that they didn't know the answer. We considered two types of sand exposure: (1) digging in the sand; and (2) having one's body buried in the sand. Participants who "dug in the sand" were defined as those who reported that they dug in the sand or built sand castles at the beach the day of the interview. Having one's body buried in the sand was defined as those who reported that they had their body buried in the sand at the beach on the day of the interview. We considered being buried in the sand a more intense sand exposure than digging in the sand.

e. Exposure Period

The exposure period encompasses recreational activities performed during the day of enrollment while participants were at the beach. This was the period between the baseline enrollment interview and the beach exit interview. The enrollment interview was conducted as participants arrived at the beach and the exit interview was conducted after completion of enrollment-day recreational activities as participants were leaving the beach. During the beach enrollment interview participants were asked about the exposures and activities that

occurred 3 days prior to enrollment and during the exit interview participants were asked about the exposures and activities that occurred on the day of enrollment, respectively. Some exposure questions were repeated during the telephone follow-up interview conducted 10-12 days after participants departed the beach.

f. Health Assessment

NEEAR water study participants were interviewed at baseline and asked to report physical health symptoms and illness experienced during the 3 days prior to enrollment. Participants who agreed were contacted by telephone 10-12 days following the day of beach exposure and asked to report if they had experienced physical symptoms of gastrointestinal illness (GI illness) since their beach-exit interview the day of enrollment. GI illness was defined as any of the following: diarrhea (three or more loose stools in a 24-hour period); vomiting; nausea and stomach ache; nausea or stomach ache, and interference with regular activities (missed time from work or school, or missed regular activities as a result of the illness).

During the telephone follow-up interview, participants answered a series of questions about the occurrence of physical symptoms and illness. Ascertainment of physical symptoms and illness at baseline (3 days prior to enrollment) and again after 10 to 12 days of follow-up allowed us to differentiate between pre-existing or prevalent health outcomes and incident health outcomes. During the telephone follow-up interview we also asked participants about other potential risk factors since the enrollment-day interview. This included information such as the number of times participants went to the same beach, went swimming at another beach (swimming defined as any water contact), went swimming in a pool, or ate raw or

under-cooked foods (e.g., red meat, fish, shellfish, eggs). Participants with prevalent GI illness were excluded from the analysis.

g. Statistical Analysis

A preliminary review of sand fecal indicator measure, sand exposure, demographic, health outcome, and covariate data was performed to check for implausible data points and outliers. Errors in data were evaluated and, if deemed actual errors based upon comparisons with other observations' values or substantive reasoning, were deleted. Analysis data sets were also cleaned and evaluated for missing values. Only observations with complete data on fecal indicator measures and important covariates (age, sex, race/ethnicity, swimming status, and beach) were kept. This involved creation of a data set that excluded missing data values for the outcome (GI illness), sand exposure variables (digging in the sand and body buried in the sand), and critical covariates (age, sex, race/ethnicity, swimming status, and beach).

1. Sand Fecal Indicator Organism Data Analysis

We first explored the spatial variability of sand fecal indicator measure data by examining the proportion of fecal indicator-positive sand samples by collection transect at each beach. Because fecal indicator organism data were highly skewed, raw data were log-transformed (base 10) for analysis. The arithmetic mean of the log-transformed values was used to summarize sand quality at the beach on a given day. F^+-specific coliphage samples below the detection limit (0.0092 PFU/g) were assigned a value of one-half the lower detection limit (0.0046 PFU/g). *Enterococcus* samples below the detection limit by Method 1600 (0 CFU/g) were assigned a value of 0.1 CFU/g to allow log-transformation of the raw

data. qPCR CCE samples below the detection limit were assigned the value of the lower detection limit and subsequently log-transformed (base 10). We focused the analyses on the daily average of the three 8:00 AM sand samples. The daily average represented average sand quality at the beach on a particular day. Analysis of variance models were used to explore the relationship between $\log_{10}$ qPCR CCE and $\log_{10}$ CFU by beach. We examined the frequency of each beach sand contact activity – digging in the sand or building sand castles; having one's body buried in the sand – first stratified by beach and then across all beaches combined.

For the fecal indicator measures, we considered the following types of exposure definitions: (1) a simple presence-absence categorical variable; (2) a categorical variable reflecting the number of samples positive out of the three samples collected each day (used only for F^+-specific coliphage); (3) a categorical variable of less than or equal to the median and above the median value; (4) a tertiles variable; and (5) a quartiles variable. (6) a continuous variable (except for F^+-specific coliphage);

2. Sand Fecal Indicator Organism Densities and GI Illness Association Data Analysis

We first considered categorical classifications of the fecal indicator measures of sand quality. Because a large proportion of F^+-specific coliphage data were below the detection limit we only considered classifications involving simple presence/absence, number of positive samples out of the 3 samples collected each day and ≤ vs. > the median concentration (PFU/g). For the bacterial fecal indicator measures (*Enterococcus* CFU/g, and *Enterococcus*, *Bacteroides*, and *B. thetaiotaomicron* qPCR CCE/g) we did not consider the classification of number of positive samples out of the 3 samples collected each day because,

unlike F^+-specific coliphage, there were few days when these indicators were not detected in beach sand. Instead, in addition to simple presence/absence and ≤ vs. > the median, we evaluated categorical classifications of tertiles (of CFU/g and qPCR CCE/g). Categorical models involved comparisons between those in contact with sand on days when there was a specific average level of a specific fecal indicator present (e.g., above the median, highest tertile) and those in contact with sand on days when that specific fecal indicator was absent. For these models, the aOR can be interpreted as the risk (odds) of GI illness among those digging in sand when a specific fecal indicator was present at a specific daily average category (e.g., highest tertile) divided by the risk (odds) of GI illness among those digging in the sand when that particular fecal indicator was absent in sand. Then we evaluated comparisons between those in contact with sand on days when a specific fecal indicator was present at a specific daily average category (e.g., highest tertile) to those who were not in contact with sand (did not dig; were not buried in sand). For these models, the aOR can be interpreted as the risk (odds) of GI illness among those who dug in the sand on days when average sand quality was at a specific level (e.g., highest tertile) divided by the risk (odds) of GI illness among those who did not have contact with sand (did not dig in the sand; were not buried in sand).

Next we evaluated the association between each continuous sand fecal indicator measure and GI illness: (1) only among those engaged in each sand contact activity (digging in the sand or building sand castles; and having one's body buried in the sand); (2) among all participants with those not engaged in each sand contact activity as the reference category; (3) among all participants with those not engaged in each sand contact activity assigned a uniformly low exposure value for each fecal indicator. We considered the body buried in the

sand exposure to be a more intense form of sand contact than digging in the sand. Using logistic regression models, we estimated adjusted odds ratios (aOR) and 95% confidence intervals (CI) for GI illness and its association with fecal indicator organism measures among those digging in the sand. For these models, the aOR can be interpreted as the increase in the odds of GI illness per unit increase in the fecal indicator organisms in the sand among those in contact with sand (digging in the sand; body buried in sand). Next we considered models where those not in contact with sand were included in the analysis, but assigned a uniformly low value of sand quality exposure - 10% and 1% of the lower detection limit for each fecal indicator measure. This was performed because we considered it possible that those not engaging in the two sand contact activities (digging in the sand; body buried in the sand) may have experienced a uniformly low exposure to beach sand through incidental contact (e.g., setting up or breaking down a beach chair or sitting on a sandy beach towel during the day). We evaluated whether the choice of different values (10% vs. 1% of the detection limit) assigned to those who reported no contact with sand affected the results of the fecal indicator-GI illness relationship.

To evaluate the occurrence of multiple fecal indicators in the sand, we considered an exploratory, non-traditional method of creating an index variable defined by the presence/absence of each of the fecal indicators at each of the 3 sample locations. The range of the fecal indicator index variable (from 0 to 15) was an indication of presence/absence for each of the five fecal indicators summed across the 3 sampling transects. This index variable was classified into categories of 0 to 4, 5 to 9, and 10 to 15. This variable was created as an alternative method to represent daily average sand quality reflecting the presence of one or more of the fecal indicator measures.

Models using an identity link and a binomial error structure (linear model) were used to directly estimate the attributable risk[142] (risk among those with sand contact minus the risk among those without sand contact) which we refer to as illness associated with each of the sand contact activities (digging in sand; buried in sand).

We assumed the household was the unit of independence in the data. There are no individual identifiers in the data (e.g., name, Social Security number, address). To identify potential re-enrollees we matched observations on age (birth date), sex, race, ZIP code, beach and several chronic, conditions (Crohn's disease, irritable bowel syndrome, asthma, emphysema / COPD, and chronic skin problems such as psoriasis or eczema). Observations that matched on all of these factors were considered potential re-enrollees. To account for the non-independence of re-enrollment, the unique household ID of the re-enrollment study entry date was re-assigned to the unique household ID of the first study entry date. Robust variance estimates were used to account for the non-independence of observations within household.[163,164]

We evaluated relationships between the exposure (beach sand contact activities), health outcomes, and covariates that were important potential confounders. We considered covariates strongly associated with beach sand contact and illness or those regarded by investigators to be potential confounding factors for inclusion in regression models. The factors included in all regression models were age, sex, race/ethnicity, swimming (defined as any contact swimming), and beach. Although information on other covariates was collected (i.e., contact with animals, contact with other persons with diarrhea, number of other visits to the beach, any other chronic illnesses (GI, skin, asthma) and eating any food or drink while at the beach) these factors were not evaluated because of the limited sample size of the 2

beaches. Because of the sparseness of the data (limited sample size) we were not able to evaluate potential effect measure heterogeneity (on the multiplicative scale) of the sand exposure/illness relationship across strata of covariates with biological plausibility as potential effect measure modifiers. We reported combined effect measure estimates adjusted for potential confounders. All analyses were completed using SAS version 9 © (SAS Institute Inc., Cary, NC, USA) and Stata version 9.2 © (StataCorp LP, College Station, TX, USA).

3. Results

A total of 4,999 interviews from 2,388 household groups were completed. Of these interviews 4,838 respondents provided complete information on age, sex, race/ethnicity, contact with beach sand, and swimming. Relationships between fecal indicator measures, sand exposure, and GI illness were evaluated using interviews with complete exposure, outcome, and covariate information.

Respondents at the 2 beaches differed by age, race/ethnicity (defined as white/non-white), miles traveled to the beach, and proportion of individuals who reported contact with the sand. Respondents were 66% white and 58% female, with a median age of 30 years. Those who dug in the sand were younger than those who did not dig in the sand (median age 10 and 36 years, respectively) but were equally likely to report vomiting and other GI symptoms at baseline (Table 1). More individuals who dug in the sand reported contact with animals 48 h prior to or immediately after the beach visit (71.3% vs. 61.5%) (Table 1). Fewer individuals who dug in the sand reported a history of chronic GI illness (1.6% vs. 3.5%) and consumption of raw or red meat prior to or immediately after the beach visit (8.4% vs. 14.4%). Digging in the sand was strongly associated with swimming as 80.9 % of

swimmers reported digging in the sand compared to only 19.2% of non-swimmers. Those who had their body buried in sand were also younger than those who did not have their body buried in the sand (median age 8 and 31 years, respectively), were equally likely to report vomiting and other GI symptoms at baseline (Table 1). Those who had their body buried in sand were less likely to report a history of chronic GI illness (0% vs. 3.1%), less likely to report consumption of raw or red meat 48 h prior to or immediately after the beach visit (6.6% vs. 13%), and more likely to report contact with animals 48 h prior to or immediately after the beach visit (68.7% vs. 63.9%). Having one's body buried in the sand was strongly associated with swimming, 88.4 % of swimmers reported being buried in the sand compared to only 11.7% of non-swimmers.

There were slightly more female beach-goers than male in both sand-contact groups. There were differences in frequency of sand contact by race/ethnicity (Table 1). Most participants in contact with sand were white. Results by race/ethnicity for body buried in the sand were similar to the exposure of digging in the sand (Table 1). Participants at Fairhope Beach were more likely to have reported digging in the sand (37.7%) and having their body buried in the sand (5.5%) (Table 1).

Concentrations of fecal indicator organisms in sand measurements differed by beach (Table 2). The mean concentration of F^+-specific coliphage was higher at Goddard Beach (1.5406 PFU/g vs. 0.14 PFU/g) as well as for each of the qPCR measures (*Enterococcus*, *Bacteroides*, and *B. thetaiotaomicron*) (Table 2). The mean concentration of the culture-based *Enterococcus* measure (CFU/g) was higher at Fairhope Beach (87.4 CFU/g vs. 32.2 CFU/g). Of all the fecal indicator measures, F^+-specific coliphage was detected least frequently with 82.9% of samples below the detection limit. For the remaining fecal

indicators the percent below the detection limit ranged from 18.0% for *Enterococcus* (qPCR CCE/g) to 52.6% for *Bacteroides* (qPCR CCE/g). We observed spatial variability in fecal indicator measures (Table 3). At Fairhope Beach a higher percentage of samples was positive at transect 3 (Table 3). At Goddard Beach a higher percentage of samples was positive at transect 1 (Table 3).

a. Relationship Between Sand Contact, Measures of Fecal Indicators in Sand, and GI Illness

The incidence of GI illness was 6.2% (301 of 4523) during the 10 to 12 day follow-up period. GI illness incidence was highest among children younger than 5 years (9.5%) and lowest among those aged 55 and older (5.5%). The adjusted risk (odds) of illness was 1.40 times higher among those who dug in the sand than among those who did not dig in the sand (95% CI = 1.02–1.93). The risk of GI illness among children aged 10 and younger who dug in the sand was 1.81 times the risk of GI illness among those who did not dig in the sand (95% CI = 0.96–3.43). The association was weaker between digging and GI illness among children and adults aged 11 to 54 (aOR = 1.26; 95% CI = 0.85–1.87) and among older adults (aOR = 1.33; 95% CI = 0.34–5.29).

Categorical classifications involving presence/absence and above/below the median value were used for the F^+-specific coliphage because there were few days when this measure was detected in sand (Table 4). The contrast of those who dug in the sand on days when F^+-specific coliphage was present compared to those who did not dig in the sand (aOR = 1.25; 95% CI = 0.72–2.16) produced a lower point estimate than the point estimate of the contrast of those who dug in the sand on days when F^+-specific coliphage was absent compared to those who did not dig in the sand (aOR = 1.57; 95% CI = 1.02–2.41) (Table 4). For the body buried in the sand exposure (which we considered a more intense exposure to sand), there

was an elevated effect estimate comparing those buried in the sand on days when F^+-specific coliphage was present to those not buried in the sand, but the point estimate was imprecise (aOR = 2.41; 95% CI = 0.78–7.42). Categorization of the number of F^+-specific coliphage-positive sand samples out of the 3 samples collected each day revealed a weak positive association. The risk (odds) of GI illness among those digging in the sand on days when 3 out of 3 samples were positive was 2.44 times the risk (odds) of GI illness among those who did not dig in the sand (95% CI = 0.87–6.87). Small sample sizes did not permit estimation of effect measures for those buried in sand using this classification method. A stronger, but imprecise point estimate was observed for the comparison of the risk (odds) of GI illness among those who were buried in the sand on days with F^+-specific coliphage above the median value (0.00124 PFU/g) to the risk (odds) of GI illness among those not buried in the sand (aOR = 4.49; 95% CI = 0.36–55.76). However, overall, these results are not suggestive of a strong association between F^+-specific coliphage, the sand exposures considered, and GI illness (Table 4).

For culturable *Enterococcus* (by Method 1600), there was a positive association with GI illness for digging in the sand, but not for the body buried in the sand exposure (Table 5). The risk (odds) of GI illness among those digging in the sand on days when daily average *Enterococcus* levels (CFU/g) were in the highest quartile was 2.00 times the risk (odds) of GI illness among those not digging in the sand (95% CI = 1.28–3.12). A weakening of this positive association was evident for the sand exposure buried in the sand (Table 5).

For *Enterococcus* measured in sand by qPCR CCE method, we observed a consistently positive association with GI illness for the digging in the sand exposure (Table 6). The risk (odds) of GI illness among those digging in the sand with *Enterococcus* in the

highest tertile (>48.87 qPCR CCE/g) was 2.20 times the risk (odds) of GI illness among those digging in the sand with *Enterococcus* in the lowest tertile (>0–28.04 qPCR CCE/g) (95% CI = 1.09–4.44). The risk (odds) of GI illness among those digging in the sand with *Enterococcus* in the highest tertile (>48.87 qPCR CCE/g) was 1.95 times the risk (odds) of GI illness among those not digging in the sand (95% CI = 1.23–3.09). Among the most highly exposed group (>48.87 qPCR CCE/g) we estimated an excess of 41 cases of GI illness per 1000 individuals compared to those who did not dig in the sand.

The magnitude of the positive association between *Enterococcus* (qPCR CCE) and GI illness increased for the more intense sand contact exposure, buried in the sand (Table 6). The risk (odds) of GI illness among those buried in the sand with *Enterococcus* in the highest tertile (>48.87 qPCR CCE/g) was 9.11 times the risk (odds) of GI illness among those buried in the sand with *Enterococcus* in the lowest tertile (>0–28.04 qPCR CCE/g) (95% CI = 1.47–56.35) (Table 6). However, the wide 95% confidence interval reflects the imprecision of this point estimate and the fact that there were few participants in this group. This observed positive association between *Enterococcus* (qPCR CCE/g) in sand, sand contact exposures, and GI illness was robust to choice of reference category. For example, the risk (odds) of GI illness among those buried in sand in the highest tertile of *Enterococcus* exposure (>48.87 qPCR CCE/g) was 3.49 times the risk (odds) of GI illness among those who did not report being buried in the sand (95% CI = 1.43–8.50) (Table 6). Among the most highly exposed group (>48.87 qPCR CCE/g) we estimated an excess of 115 cases of GI illness per 1000 individuals compared to those who were not buried in the sand.

An inconsistent relationship was observed between *Bacteroides* (qPCR CCE/g) and GI illness for each of the sand exposures (Table 7). Although positive associations were

observed between *Bacteroides* (qPCR CCE/g) and GI illness for the classification of above/below the median for both digging in the sand (aOR = 1.80; 95% CI = 1.21–2.69) and buried in sand (aOR = 2.90; 95% CI = 1.40–6.00), these positive associations were not robust to the other classification methods that were considered (presence/absence, tertiles, and quartiles) (Table 7). For example, a decline was observed among those with digging exposure in the highest quartile of *Bacteroides* in sand (>241.31 qPCR CCE/g) (aOR = 1.17; 95% CI = 0.67–2.07) (Table 7). A similarly inconsistent relationship was observed between *B. thetaiotaomicron* (qPCR CCE/g) (considered to be a more human-specific indicator of fecal contamination) and GI illness for both sand contact exposures across categorical classification methods (Table 8).

The incidence of GI illness was positively associated with densities of *Enterococcus* qPCR CCE in sand for both forms of sand contact considered (Table 9). Among participants who reported digging in the sand, a 1 $\log_{10}$ increase in the daily *Enterococcus* qPCR CCE average resulted in a 1.45 increase in the risk (odds) of GI illness (95% CI = 1.05–2.01). The relationship was stronger among participants buried in the sand (aOR = 3.12; 95% CI = 1.08–9.05). Re-assignment of a uniformly low value to those not in contact with sand (i.e., not digging; not buried) resulted in a decrease in the magnitude of observed associations, but an improvement in precision (Table 9). After re-assigning those not digging in the sand a uniformly low value of exposure, a 1 $\log_{10}$ increase in the daily *Enterococcus* qPCR CCE average resulted in a 1.11 increase in the risk (odds) of GI illness (95% CI = 1.03–1.18). The choice of uniformly low value (10% or 1% of the daily average detection limit) for re-assignment did not substantially alter the results (data not shown).

The relationship between the remaining fecal indicator measures (culture-based *Enterococcus*, and qPCR CCE *Bacteroides* and *B. thetaiotaomicron*), respectively with GI illness for the two sand contact exposures was not as strong or consistent as the association observed for the *Enterococcus* qPCR CCE measure (Table 9). Although positive relationships were observed for both *Enterococcus* CFU as well as *Bacteroides* QPCR CCE densities. For the associations between these remaining continuous fecal indicator measures in sand and GI illness, the aORs ranged from 0.95 to 1.33 for digging in the sand and from 1.11 to 1.65 for buried in the sand (Table 9). We did not report model estimates of the F^+-specific coliphage continuous measure because there were so few days when F^+-specific coliphage was detected, leading to unstable and imprecise estimates.

The composite index of fecal contamination showed consistent and positive associations with GI illness. This was observed by classification of the 5 fecal indicators into an index with categories of positive samples summed across each of the 3 sand samples that were collected at the 2 marine beaches each day (Table 10). This relationship was robust to type of sand contact (digging in sand; body buried in the sand). The risk (odds) of GI illness among those digging in the sand on days when 10-15 out of the 15 sand samples were positive for fecal indicators was 1.69 times the risk (odds) of GI illness among those not digging in the sand (95% CI = 1.03–2.78). The magnitude of the positive association was greater for the more intense exposure buried in the sand. The risk (odds) of GI illness among those buried in the sand on days when 10-15 out of the 15 sand samples were positive for fecal indicators was 2.84 times the risk (odds) of GI illness among those not buried in the sand (95% CI = 1.01–7.97). For those buried in the sand, the association was robust to choice of reference category. The risk (odds) of GI illness among those buried in the sand on

days when 10-15 out of the 15 sand samples were positive for fecal indicators was 5.48 times the risk (odds) of GI illness among those buried in the sand on days when 0-4 out of the 15 sand samples were positive for fecal indicators (95% CI = 1.09–27.56).

4. Discussion

This investigation evaluated associations between 5 fecal indicators measured daily in sand along 3 transects at 2 marine beaches, two types of sand contact activities (digging in sand; buried in sand), and GI illness. A molecular measure of fecal contamination (*Enterococcus* qPCR CCE) was consistently associated with GI illness for both sand contact activities. The strong positive association observed between daily average concentrations of *Enterococcus* (qPCR CCE/g) in sand, sand contact exposures, and GI illness was robust to the various estimation approaches that were considered – continuous variable models vs. categorical variable models (including use of various reference categories). There was moderate evidence of positive associations between the 2 sand contact activities and GI illness for culturable *Enterococcus*, culturable F^+-specific coliphage, and molecular *Bacteroides*, however, these fecal indicator measures showed some inconsistency with respect to the methods of exposure classification considered. Our ability to make conclusions for the F^+-specific coliphage measure was limited because of the low percentage (17.1%) of days when it was detected in beach sand samples (Table 2). *B. thetaiotaomicron* did not show a consistent or strong association with GI illness for the 2 sand exposures considered. An index variable of the daily sum of all 5 fecal indicators present or absent in beach sand at the 3 transects demonstrated a consistently positive association with GI illness. This index reflects both the diversity and presence of multiple fecal indicators in sand on a given day. This index provides some evidence that the presence of one or more fecal

indicators in the sand on a given day may be associated with an increased risk of GI illness among those exposed. However, this index does not account for a specific combination of fecal indicators that may be present in sand and should be viewed as an index of the diversity of multiple fecal indicators to assess daily sand quality for exposure classification.

To the best of our knowledge, this is the first study to demonstrate an association between GI illness and beach sand contact as a function of microbial sand quality. One previous study demonstrated a relationship between sand contact and GI illness; however it observed a positive association with GI illness as a function of time spent exposed to wet sand, not as a function of microbial sand quality.[41] The results of two other studies of beach sand exposure and health effects (which included beach sand fecal indicator measures) did not demonstrate an association between fecal contamination in beach sand, sand contact activities, and illness (including GI illness).[32,124] These investigators did observe higher concentrations of fecal microbial indicators (including *E. coli* and *Enterococcus*) in beach sand/sediment samples compared to beach water samples. It has been hypothesized that sand could serve as a source of fecal contamination in water, especially in the surf zone along the shoreline. Numerous exposure assessments have demonstrated that sand can serve as a both a source and a sink of fecal microbial contamination. Debate therefore exists concerning use of fecal indicator bacteria (*E. coli* and *Enterococcus*) to monitor beach water quality and sand quality. Several studies have demonstrated potential for re-growth of *E. coli* and *Enterococcus* in sand; further complicating a clear understanding of fecal contamination sources. Beach sand may serve as a source of autochthonous *E. coli* and *Enterococcus* in water in the absence of fecal contamination (and associated pathogens) and contribute to unnecessary beach closures when relying on water tests of fecal indicators bacteria.[68,69] EPA

guidelines for monitoring of fresh and marine recreational waters are based on *E. coli* and *Enterococcus* and currently, no sand quality guidelines exist.

Although there is debate about the relative impact of autochthonous fecal indicator populations in sand versus non-point source (urban run-off, domestic and wild animal, and human bather) versus point source (municipal sewage outfalls) fecal contamination on sand quality, our results are suggestive of an association between *Enterococcus* qPCR CCE and GI illness. This association was stronger than that observed for the traditional culturable measure of *Enterococcus* and several novel measures (culturable F^+-specific coliphage, and molecular *Bacteroides* and *B. thetaiotaomicron*) that are thought to be better indicators of human-specific fecal contamination. Previous research has demonstrated a strong and consistent association between *Enterococcus* qPCR CCE and swimming-associated illness among adults and also among children.[9,146] Wade et al., speculated that "molecular measurement of *Enterococcus* DNA provides a stable, conservative means of quantifying the level of fecal contamination, which is not subject to die-off but may mirror the dilution and dispersion of fecal material.[146]" Culturable fecal indicator bacteria cells (e.g., total and fecal coliforms, *E. coli*, and *Enterococcus*) are readily inactivated during the sewage treatment process whereas pathogenic viruses and protozoan parasites often survive treatment and are discharged into recreational waters. Molecular methods of measuring *Enterococcus* may provide a more reliable estimate of pathogenic microbes that survive wastewater treatment and are discharged into the water environment.

Water quality at the 2 marine beaches studied was influenced by human sources of pollution (nearby municipal sewage outfall). It is possible that fecal contamination from municipal sewage outfalls reached the shoreline producing impacts on the beach environment

as well as sand quality through tidal flow, wave action, and currents. There is, however, contradictory evidence suggesting that sewage from municipal outfalls does not predominate the beach environment as much as diffuse fecal contamination sources (such as coastal birds, other animal populations, and urban run-off).[81,180,181] We do not know if the relationships we observed between *Enterococcus* qPCR CCE and GI illness can be extended to beaches not influenced by a municipal sewage outfall (i.e., beaches primarily influenced by non-point source pollution) or to freshwater beaches.

The definition of GI illness in this study was non-specific, and may have been affected by recall bias. A broad endpoint accounted for the diverse range of GI symptoms potentially associated with sand exposure, but may obscure more specific effects of sand quality and sand exposure. While those in contact with sand (digging in sand; buried in sand) may have been more likely to recall illness than those not in contact with sand, it is unlikely that recall occurred among those in contact with sand at varying levels of sand quality. The primary focus of the NEEAR water studies is water quality and swimming-associated illness, also making it unlikely that participants in contact with sand would have been more likely to recall illness than those not in contact with sand. Digging in the sand (80.9 % of swimmers vs. 19.2% of nonswimmers reported digging in sand) and being buried in the sand (88.6% of swimmers vs. 11.4% of nonswimmers reported being buried in sand) were strongly associated with swimming. This may make it difficult to tease out the effect of sand exposure alone. However, consistency in the associations between *Enterococcus* qPCR CCE and GI illness was observed across varying definitions of sand contact (digging in the sand, buried in the sand). As was expected, for a more intense sand exposure (those buried in the sand), the results observed were stronger than the results for those digging in sand. We

also considered a definition of sand contact defined as any sand contact (either digging in the sand or buried in the sand) and the results for this definition did not substantially alter the results (data not shown).

5. Conclusions

Research on health effects among beach-goers has largely focused on investigating swimming-associated illness and microbial water quality and has neglected relationships between sand contact activities, sand quality, and health effects. As far as we know, this is the first study to demonstrate a relationship between sand contact activities and GI illness as a function of microbial sand quality. Further investigation of the microbial quality of beach sand and its association with enteric and non-enteric illness among those in contact with sand appears warranted based on our results. It is unknown whether the relationships we observed between *Enterococcus* qPCR CCE and GI illness can be extended to more specific definitions of GI illness (diarrhea, vomiting) and nonenteric illnesses (upper respiratory illness, eye irritation, skin rash, earache, and infected cuts/wounds). Furture studies should consider the relationship between sand quality and health effects among those in contact with sand. Confirmation of the findings presented here at a broader geographic range of sites and sand types will help to further understanding of illness risks associated with sand exposure and the association between illness and fecal indicator organisms in the sand.

Table 1. Characteristics of Those Who Did Not Dig in the Sand, Those Who Dug in the Sand, Those Who Did Not Have Their Body Buried in the Sand, and Those Who Did Have Their Body Buried in the Sand.

	Digging in the Sand		Body Buried in the Sand	
	No (n = 3512)	Yes (n = 1326)	No (n = 4627)	Yes (n=211)
Characteristic	No. (%)[a]	No. (%)[a]	No. (%)[a]	No. (%)[a]
Age (yrs)				
0-4	179 (5.1)	287 (21.6)	418 (9.0)	48 (22.7)
5-10	208 (5.9)	411 (31.0)	529 (11.4)	90 (42.7)
11-19	348 (9.9)	165 (12.4)	493 (10.7)	20 (9.5)
20-54	2142 (61.0)	427 (32.2)	2518 (54.4)	51 (24.2)
55+	635 (18.1)	36 (2.7)	669 (14.5)	2 (<1)
Sex				
Male	1471 (41.9)	584 (44.0)	1961 (42.4)	94 (44.6)
Female	2041 (58.1)	742 (56.0)	2666 (57.6)	117 (55.4)
Race				
White	2315 (65.9)	870 (65.6)	3061 (66.2)	124 (58.8)
Black	467 (13.3)	222 (16.7)	653 (14.1)	36 (17.1)
Asian	60 (1.7)	24 (1.8)	82 (1.8)	2 (<1)
American Indian	7 (<1)	5 (<1)	9 (<1)	3 (1.4)
Hispanic/Latino	636 (18.1)	186 (14.0)	778 (16.8)	44 (20.9)
Multiethnic/other	27 (<1)	19 (1.4)	44 (1.0)	2 (1.0)
Miles traveled to the beach				
0-5 miles	851 (24.7)	320 (24.5)	1117 (24.6)	54 (26.1)
6-20 miles	1556 (45.2)	504 (38.6)	1981 (43.6)	79 (38.2)
21-50 miles	781 (22.7)	394 (30.2)	1108 (24.4)	67 (32.4)
50 miles or greater	254 (7.4)	89 (6.8)	336 (7.4)	7 (3.4)
Conditions in the 3 d prior to the beach visit				
Vomiting	49 (1.4)	22 (1.7)	66 (1.4)	5 (2.4)
Other GI symptoms	99 (2.8)	30 (2.3)	125 (2.7)	4 (1.9)
Sore throat	153 (4.4)	80 (6.0)	223 (4.8)	10 (4.7)
Rash	77 (2.2)	49 (3.7)	114 (2.5)	12 (5.7)
Sunburn	159 (4.5)	40 (3.0)	195 (4.2)	4 (1.9)
Infected cut	185 (5.3)	139 (10.5)	204 (6.6)	20 (9.5)
Eye irritation	20 (<1)	9 (<1)	29 (<1)	0 (0)
Earache	48 (1.4)	19 (1.4)	64 (1.4)	3 (1.4)
History of chronic respiratory problems or asthma	237 (6.8)	87 (6.6)	313 (6.8)	11 (5.2)
History of allergies	606 (17.3)	197 (14.9)	779 (16.8)	24 (11.4)
History of chronic GI illness	124 (3.5)	21 (1.6)	145 (3.1)	0 (0)
Any history of chronic GI illness, asthma, or allergies	606 (17.3)	197 (14.9)	779 (16.8)	24 (11.4)
Water contact status				
No water contact	2117 (60.3)	254 (19.2)	2347 (50.7)	24 (11.4)
Water contact	1395 (39.7)	1072 (80.8)	2280 (49.3)	187 (88.6)
Contact with animals 48 h prior to or after beach visit, or between beach visit and phone interview	2160 (61.5)	945 (71.3)	2960 (63.9)	145 (68.7)
Consumption of raw meat 48 h prior to beach visit or between beach visit and phone interview	506 (14.4)	111 (8.4)	603 (13.0)	14 (6.6)
Beach				
Fairhope Beach	1242 (35.4)	752 (56.7)	1884 (40.7)	110 (52.1)
Goddard Beach	2270 (64.6)	574 (43.3)	2743 (59.3)	101 (47.9)

[a]Excludes those with missing information on age, sex, race/ethnicity, water contact status, and beach.

TABLE 2. Descriptive Statistics of Fecal Indicator Measures in Sand by Beach.

	F^+-Specific Coliphage (PFU/g)							
Beach	**No.**	**Mean (SD)**	**Min**	**25th Percentile**	**Median**	**75th Percentile**	**Max**	**Below lower DL No. (%)**
All beaches	123	0.0345 (0.1563)	0	0	0	0	1.5406	102 (82.9)
Fairhope Beach	51	0.0143 (0.0284)	0	0	0	0	0.14	39 (76.5)
Goddard Beach	72	0.0488 (0.2022)	0	0	0	0	1.5406	63 (87.5)
	***Enterococcus* (CFU/g)**							
Beach	**No.**	**Mean (SD)**	**Min**	**25th Percentile**	**Median**	**75th Percentile**	**Max**	**Below lower DL No. (%)**
All beaches	144	59.8 (368.2)	0	0	3.2	16.8	4160	40 (27.8)
Fairhope Beach	72	87.4 (493.3)	0	1.6	7.2	20.4	4160	13 (18.1)
Goddard Beach	72	32.2 (167.8)	0	0	1.6	6.4	1408	27 (37.5)
	***Enterococcus* (QPCR CCE/g)**							
Beach	**No.**	**Mean (SD)**	**Min**	**25th Percentile**	**Median**	**75th Percentile**	**Max**	**Below lower DL No. (%)**
All beaches	133	405.9 (2161)	0.03	4.4	24.7	73.4	20586	40 (27.8)
Fairhope Beach	61	239.7 (1617)	0.03	0.4	8.8	46.7	12658	13 (18.1)
Goddard Beach	72	546.8 (2535)	0.1	17.2	39.8	90.4	20586	27 (37.5)
	***Bacteroides* (QPCR CCE/g)**							
Beach	**No.**	**Mean (SD)**	**Min**	**25th Percentile**	**Median**	**75th Percentile**	**Max**	**Below lower DL No. (%)**
All beaches	133	477.3 (1820)	0.03	0.6	1.5	179.7	15457	70 (52.6)
Fairhope Beach	61	249.9 (903.9)	0.03	0.5	0.9	94.1	5206	38 (62.3)
Goddard Beach	72	669.9 (2321)	0.3	0.8	16.4	296.7	15457	32 (44.4)
	***B. thetaiotaomicron* (QPCR CCE/g)**							
Beach	**No.**	**Mean (SD)**	**Min**	**25th Percentile**	**Median**	**75th Percentile**	**Max**	**Below lower DL No. (%)**
All beaches	132	1207 (5329)	0.1	0.8	182.1	689.3	56829	48 (36.4)
Fairhope Beach	60	279.5 (588.5)	0.1	0.6	55.4	386	3702.6	26 (43.3)
Goddard Beach	72	1980 (7127)	0.3	1.3	436.6	1323.6	56829	22 (30.6)

TABLE 3. Spatial Variability of Fecal Indicator-Positive Samples at Each Collection Transect by Beach.

	Fairhope Beach			Goddard Beach		
Indicator	**Transect 1 No. + (%)**	**Transect 2 No. + (%)**	**Transect 3 No. + (%)**	**Transect 1 No. + (%)**	**Transect 2 No. + (%)**	**Transect 3 No. + (%)**
F+-specific coliphage (Method 1601)	4 (23.5)	1 (5.9)	7 (41.2)	2 (8.3)	2 (8.3)	5 (20.8)
Enterococcus (Method 1600)	19 (26.4)	19 (26.4)	21 (29.2)	18 (25)	15 (20.8)	12 (16.7)
Enterococcus (QPCR CCE Method)	13 (68.4)	13 (65.0)	15 (68.2)	22 (91.7)	23 (95.8)	23 (95.8)
Bacteroides (QPCR CCE Method)	6 (31.6)	8 (40.0)	9 (40.9)	15 (62.5)	16 (66.7)	9 (37.5)
B. thetaiotaomicron (QPCR CCE Method)	13 (68.4)	9 (45.0)	12 (57.1)	16 (75.0)	17 (70.8)	15 (62.5)

TABLE 4. Relationship Between F^+-Specific Coliphage (PFU/g) in Sand and GI Illness by Status of Sand Contact and by Categorical Classification.

	GI Illness			
Digging Status (Classification)	**No No. (%)**	**Yes No. (%)**	**aOR (95% CI)[a]**	**aOR (95% CI)[a]**
No	2772 (95.13)	142 (4.87)	1.00[b]	N/A
Yes (F^+-specific coliphage (F^+) absent)	586 (91.14)	57 (8.86)	1.57 (1.02-2.41)	1.00[c]
Yes (F^+ -specific coliphage (F^+) present)	289 (91.75)	26 (8.25)	1.25 (0.72-2.16)	0.73 (0.38-1.41)
Yes (1 or 2 of 3 samples F^+ positive)	257 (92.45)	21 (7.55)	1.10 (0.60-2.03)	0.63 (0.31-1.29)
Yes (3 of 3 samples F^+ positive)	32 (86.49)	5 (13.51)	2.44 (0.87-6.87)	1.64 (0.56-4.78)
Yes (F^+ ≤ median of 0.0124 PFU/g)	171 (91.94)	15 (8.06)	1.30 (0.68-2.51)	0.81 (0.38-1.69)
Yes (F^+ > median of 0.0124 PFU/g)	118 (91.47)	11 (8.53)	1.17 (0.49-2.81)	0.64 (0.24-1.67)
	GI Illness			
Buried Status (Classification)	**No No. (%)**	**Yes No. (%)**	**aOR (95% CI)[a]**	**aOR (95% CI)[a]**
No	3508 (94.4)	208 (5.6)	1.00[b]	N/A
Yes (F^+-specific coliphage (F^+) absent)	100 (91.74)	9 (8.26)	1.55 (0.70-3.43)	1.00[c]
Yes (F^+ -specific coliphage (F^+) present)	42 (84)	8 (16)	2.41 (0.78-7.42)	1.58 (0.49-5.03)
Yes (1 or 2 of 3 samples F^+ positive)	42 (84)	8 (16)	2.41 (0.78-7.42)	1.58 (0.49-5.03)
Yes (3 of 3 samples F^+ positive)	0 (0)	0 (0)	-	-
Yes (F^+ ≤ median of 0.0124 PFU/g)	35 (87.5)	5 (12.5)	1.91 (0.56-6.50)	1.24 (0.35-4.35)
Yes (F^+ > median of 0.0124 PFU/g)	7 (70)	3 (30)	4.49 (0.36-55.76)	3.72 (0.29-48.30)

[a]Robust variance estimates clustering on household. [b]Those who did not report the sand contact activity (digging; buried) are the reference category. [c]Those who reported the sand contact activity (digging; buried) with F^+-specific coliphage absent are the reference category. aOR estimated from logistic regression model adjusted for age, sex, race/ethnicity, beach, and anycontact swimming.

TABLE 5. Relationship Between *Enterococcus* (CFU/g) in Sand and GI Illness by Status of Sand Contact and by Categorical Classification.

	GI Illness			
	No	**Yes**		
Digging Status (Categorical Classification)	**No. (%)**	**No. (%)**	**aOR (95% CI)[a]**	**aOR (95% CI)[a]**
No	3247 (94.69)	182 (5.31)	1.00[b]	N/A
Yes (*Enterococcus* (ENT) absent)	85 (97.7)	2 (2.3)	0.48 (0.12-2.00)	1.00[c]
Yes (*Enterococcus* (ENT) present)	1084 (90.79)	110 (9.21)	1.50 (1.08-2.07)	3.14 (0.72-13.69)
Yes (ENT ≤ median of 11.23 CFU/g)	670 (93.18)	49 (6.82)	1.26 (0.85-1.87)	1.00[d]
Yes (ENT > median of 11.23 CFU/g)	499 (88.79)	63 (11.21)	1.64 (1.08-2.48)	1.31 (0.81-2.11)
Yes (ENT tertile 1: >0 - 4.26 CFU/g)	383 (93.19)	28 (6.81)	1.35 (0.84-2.17)	1.00[d]
Yes (ENT tertile 2: >4.26 - 22.4 CFU/g)	408 (90.87)	41 (9.13)	1.40 (0.87-2.27)	0.99 (0.50-1.98)
Yes (ENT tertile 3: >22.4 - 1390.4 CFU/g)	378 (89.79)	43 (10.21)	1.53 (0.97-2.42)	1.14 (0.60-2.15)
Yes (ENT quartile 1: >0 - 2.96 CFU/g)	309 (93.35)	22 (6.65)	1.36 (0.79-2.32)	1.00[d]
Yes (ENT quartile 2: >2.96 - 11.23 CFU/g)	318 (92.71)	25 (7.29)	1.34 (0.68-1.89)	0.79 (0.36-1.73)
Yes (ENT quartile 3: >11.23 - 35.46 CFU/g)	242 (91.67)	22 (8.33)	1.20 (0.61-2.35)	0.79 (0.32-1.91)
Yes (ENT quartile 4: > 35.46- 1390.4 CFU/g)	300 (87.46)	43 (12.54)	2.00 (1.28-3.12)	1.45 (0.73-2.87)
	GI Illness			
	No	**Yes**		
Buried Status (Categorical Classification)	**No. (%)**	**No. (%)**	**aOR (95% CI)[a]**	**aOR (95% CI)[a]**
No	4229 (93.92)	274 (6.08)	1.00[b]	N/A
Yes (*Enterococcus* (ENT) absent)	21 (87.5)	3 (12.5)	3.06 (0.81-11.52)	1.00[c]
Yes (*Enterococcus* (ENT) present)	169 (90.86)	17 (9.14)	1.33 (0.67-2.61)	0.37 (0.07-2.07)
Yes (ENT ≤ median of 11.23 CFU/g)	116 (92.06)	10 (7.94)	1.40 (0.68-2.87)	1.00[d]
Yes (ENT > median of 11.23 CFU/g)	74 (88.10)	10 (11.90)	1.52 (0.58-4.03)	1.39 (0.44-4.37)
Yes (ENT tertile 1: >0 - 4.26 CFU/g)	72 (92.31)	6 (7.69)	1.38 (0.57-3.37)	1.00[d]
Yes (ENT tertile 2: >4.26 - 22.4 CFU/g)	61 (91.04)	6 (8.96)	1.20 (0.37-3.87)	0.91 (0.22-3.77)
Yes (ENT tertile 3: >22.4 - 1390.4 CFU/g)	53 (87.69)	8 (12.31)	1.81 (0.66-4.97)	1.56 (0.36-6.73)
Yes (ENT quartile 1: >0 - 2.96 CFU/g)	34 (94.44)	2 (5.56)	0.96 (0.23-3.99)	1.00[d]
Yes (ENT quartile 2: >2.96 - 11.23 CFU/g)	43 (97.73)	1 (2.27)	0.34 (0.05-2.61)	0.12 (0.01-1.87)
Yes (ENT quartile 3: >11.23 - 35.46 CFU/g)	38 (86.36)	6 (13.64)	1.65 (0.49-5.53)	0.70 (0.58-8.44)
Yes (ENT quartile 4: > 35.46- 1390.4 CFU/g)	54 (87.10)	8 (12.90)	1.93 (0.70-5.31)	0.84 (0.12-6.06)

[a]Robust variance estimates clustering on household. [b]Those who did not report the sand contact activity (digging; buried) are the reference category. [c]Those who reported the sand contact activity (digging; buried) with *Enterococcus* absent are the reference category. [d]Those who reported the sand contact activity (digging; buried) with *Enterococcus* present at the lowest concentration category are the reference category. aOR estimated from logistic regression model adjusted for age, sex, race/ethnicity, beach, and anycontact swimming.

TABLE 6. Relationship Between *Enterococcus* (QPCR CCE/g) in Sand and GI Illness by Status of Sand Contact and by Categorical Classification.

	GI Illness			
Digging Status (Categorical Classification)	**No No. (%)**	**Yes No. (%)**	**aOR (95% CI)[a]**	**aOR (95% CI)[a]**
No	3127 (94.67)	176 (5.33)	1.00[b]	N/A
Yes (*Enterococcus* (ENT) absent)	174 (90.63)	18 (9.38)	1.15 (0.62-2.13)	1.00[c]
Yes (*Enterococcus* (ENT) present)	907 (90.79)	92 (9.21)	1.55 (1.10-2.18)	1.40 (0.72-2.71)
Yes (ENT ≤ median of 40.5 QPCR CCE/g)	549 (90.44)	58 (9.56)	1.31 (0.85-1.99)	1.00[d]
Yes (ENT > median of 40.5 QPCR CCE/g)	532 (91.10	52 (8.90)	1.68 (1.10-2.55)	1.45 (0.76-2.77)
Yes (ENT tertile 1: >0 - 28.04 QPCR CCE/g)	378 (92.20)	32 (7.80)	0.99 (0.59-1.66)	1.00[d]
Yes (ENT tertile 2: >28.04 - 48.87 QPCR CCE/g)	371 (90.49)	39 (9.51)	1.63 (1.02-2.60)	1.71 (0.90-3.26)
Yes (ENT tertile 3: >48.87 - 8981.69 QPCR CCE/g)	332 (89.49)	39 (10.51)	1.95 (1.23-3.09)	2.20 (1.09-4.44)
Yes (ENT quartile 1: >0 - 16.87 QPCR CCE/g)	261 (94.64)	22 (7.77)	0.85 (0.48-1.52)	1.00[d]
Yes (ENT quartile 2: >16.87 - 40.5 QPCR CCE/g)	331 (91.69)	30 (8.31)	1.37 (0.83-2.26)	1.75 (0.88-3.47)
Yes (ENT quartile 3: >40.5 - 115.86 QPCR CCE/g)	203 (87.88)	28 (12.12)	2.21 (1.24-3.94)	2.86 (1.29-6.34)
Yes (ENT quartile 4: >115.86 - 8981.69 QPCR CCE/g)	286 (90.51)	30 (9.49)	1.82 (1.10-3.00)	2.61 (1.20-5.67)
	GI Illness			
Buried Status (Categorical Classification)	**No No. (%)**	**Yes No. (%)**	**aOR (95% CI)[a]**	**aOR (95% CI)[a]**
No	4038 (93.82)	266 (6.18)	1.00[b]	N/A
Yes (*Enterococcus* (ENT) absent)	31 (96.88)	1 (3.13)	0.29 (0.39-2.11)	1.00[c]
Yes (*Enterococcus* (ENT) present)	142 (88.2)	19 (11.8)	2.02 (1.08-3.76)	9.46 (1.02-88.12)
Yes (ENT ≤ median of 40.5 QPCR CCE/g)	82 (94.25)	5 (5.71)	0.66 (0.27-1.64)	1.00[d]
Yes (ENT > median of 40.5 QPCR CCE/g)	91 (85.85)	15 (14.15)	2.77 (1.33-5.76)	7.22 (1.46-35.71)
Yes (ENT tertile 1: >0 - 28.04 QPCR CCE/g)	60 (93.75)	4 (6.25)	0.69 (0.25-1.94)	1.00[d]
Yes (ENT tertile 2: >28.04 - 48.87 QPCR CCE/g)	63 (92.65)	5 (7.35)	1.24 (0.49-3.11)	2.41 (0.49-11.97)
Yes (ENT tertile 3: >48.87 - 8981.69 QPCR CCE/g)	50 (81.97)	11 (18.03)	3.49 (1.43-8.50)	9.11 (1.47-56.35)
Yes (ENT quartile 1: >0 - 16.87 QPCR CCE/g)	40 (93.02)	3 (6.98)	0.68 (0.22-2.14)	1.00[d]
Yes (ENT quartile 2: >16.87 - 40.5 QPCR CCE/g)	57 (90.48)	6 (9.52)	1.53 (0.62-3.77)	3.22 (0.73-14.07)
Yes (ENT quartile 3: >40.5 - 115.86 QPCR CCE/g)	28 (90.32)	3 (9.68)	1.79 (0.24-13.31)	3.78 (0.27-53.91)
Yes (ENT quartile 4: >115.86 - 8981.69 QPCR CCE/g)	48 (85.71)	8 (14.29)	2.71 (1.06-6.94)	9.14 (1.22-68.70)

[a]Robust variance estimates clustering on household. [b]Those who did not report the sand contact activity (digging; buried) are the reference category. [c]Those who reported the sand contact activity (digging; buried) with *Enterococcus* absent are the reference category. [d]Those who reported the sand contact activity (digging; buried) with *Enterococcus* present at the lowest concentration category are the reference category. aOR estimated from logistic regression model adjusted for age, sex, race/ethnicity, beach, and anycontact swimming.

TABLE 7. Relationship Between *Bacteroides* (QPCR CCE/g) in Sand and GI Illness by Status of Sand Contact and by Categorical Classification.

	GI Illness			
	No	**Yes**		
Digging Status (Categorical Classification)	**No. (%)**	**No. (%)**	**aOR (95% CI)[a]**	**aOR (95% CI)[a]**
No	3127 (94.67)	176 (5.33)	1.00[b]	N/A
Yes (*Bacteroides* (BACT) absent)	269 (89.07)	33 (10.93)	1.58 (0.97-2.56)	1.00[c]
Yes (*Bacteroides* (BACT) present)	812 (91.34)	77 (8.66)	1.43 (1.00-2.06)	0.92 (0.53-1.60)
Yes (BACT ≤ median of 104.40 QPCR CCE/g)	561 (91.67)	51 (8.33)	1.17 (0.77-1.78)	1.00[d]
Yes (BACT > median of 104.40 QPCR CCE/g)	520 (89.81)	59 (10.19)	1.80 (1.21-2.69)	1.57 (0.93-2.66)
Yes (BACT tertile 1: >0 - 32.52 QPCR CCE/g)	393 (90.55)	41 (9.45)	1.47 (0.95-2.28)	1.00[d]
Yes (BACT tertile 2: >32.52 - 192.42 QPCR CCE/g)	328 (90.86)	33 (9.14)	1.35 (0.81-2.25)	0.91 (0.50-1.66)
Yes (BACT tertile 3: >192.42 - 6020.26 QPCR CCE/g)	360 (90.91)	36 (9.09)	1.57 (0.96-2.57)	1.06 (0.58-1.96)
Yes (BACT quartile 1: >0 - 10.57 QPCR CCE/g)	312 (90.96)	31 (9.04)	1.37 (0.84-2.21)	1.00[d]
Yes (BACT quartile 2: >10.57 - 104.40 QPCR CCE/g)	278 (91.15)	27 (8.85)	1.45 (0.87-2.43)	1.07 (0.56-2.03)
Yes (BACT quartile 3: >104.40 - 241.31 QPCR CCE/g)	186 (85.71)	31 (14.29)	2.11 (1.16-3.82)	1.51 (0.75-3.06)
Yes (BACT quartile 4: >241.31 - 6020.26 QPCR CCE/g)	305 (93.56)	21 (6.44)	1.17 (0.67-2.07)	0.86 (0.43-1.74)
	GI Illness			
	No	**Yes**		
Buried Status (Categorical Classification)	**No. (%)**	**No. (%)**	**aOR (95% CI)[a]**	**aOR (95% CI)[a]**
No	4038 (93.82)	266 (6.18)	1.00[b]	N/A
Yes (*Bacteroides* (BACT) absent)	39 (92.86)	3 (7.14)	0.75 (0.24-2.30)	1.00[c]
Yes (*Bacteroides* (BACT) present)	134 (88.74)	17 (11.26)	1.92 (0.97-3.81)	3.05 (0.71-12.93)
Yes (BACT ≤ median of 104.40 QPCR CCE/g)	89 (95.70)	4 (4.30)	0.54 (0.21-1.43)	1.00[d]
Yes (BACT > median of 104.40 QPCR CCE/g)	84 (84)	16 (16)	2.90 (1.40-6.00)	6.26 (1.74-22.47)
Yes (BACT tertile 1: >0 - 32.52 QPCR CCE/g)	65 (95.59)	3 (4.41)	0.58 (0.18-1.83)	1.00[d]
Yes (BACT tertile 2: >32.52 - 192.42 QPCR CCE/g)	49 (85.96)	8 (14.04)	2.36 (0.89-6.27)	4.03 (0.87-18.65)
Yes (BACT tertile 3: >192.42 - 6020.26 QPCR CCE/g)	59 (86.76)	9 (13.24)	2.13 (0.85-5.34)	4.10 (0.93-18.06)
Yes (BACT quartile 1: >0 - 10.57 QPCR CCE/g)	46 (95.83)	2 (4.17)	0.50 (0.13-1.97)	1.00[d]
Yes (BACT quartile 2: >10.57 - 104.40 QPCR CCE/g)	56 (90.32)	6 (9.68)	1.54 (0.66-3.61)	2.80 (0.53-14.66)
Yes (BACT quartile 3: >104.40 - 241.31 QPCR CCE/g)	17 (70.83)	7 (29.17)	5.16 (1.55-17.12)	11.89 (1.93-73.10)
Yes (BACT quartile 4: >241.31 - 6020.26 QPCR CCE/g)	54 (91.53)	5 (8.47)	1.39 (0.45-4.32)	2.94 (0.58-14.78)

[a]Robust variance estimates clustering on household. [b]Those who did not report the sand contact activity (digging; buried) are the reference category. [c]Those who reported the sand contact activity (digging; buried) with *Bacteroides* absent are the reference category. [d]Those who reported the sand contact activity (digging; buried) with *Bacteroides* present at the lowest concentration category are the reference category. aOR estimated from logistic regression model adjusted for age, sex, race/ethnicity, beach, and anycontact swimming.

TABLE 8. Relationship Between *B. thetaiotaomicron* (QPCR CCE/g) in Sand and GI Illness by Status of Sand Contact and by Classification Category.

	GI Illness			
Digging Status (Classification Category)	**No No. (%)**	**Yes No. (%)**	**aOR (95% CI)[a]**	**aOR (95% CI)[a]**
No	3060 (94.62)	174 (5.38)	1.00[b]	N/A
Yes (*B. thetaiotaomicron* (THETA) absent)	270 (91.53)	25 (8.49)	1.16 (0.70-1.93)	1.00[c]
Yes (*B. thetaiotaomicron* (THETA) present)	764 (90.95)	76 (9.05)	1.45 (0.99-2.11)	1.25 (0.69-2.26)
Yes (THETA ≤ median of 315.12 QPCR CCE/g)	586 (89.88)	66 (10.12)	1.54 (1.05-2.26)	1.00[d]
Yes (THETA > median of 315.12 QPCR CCE/g)	448 (92.75)	35 (7.25)	1.11 (0.68-1.80)	0.71 (0.41-1.24)
Yes (THETA tertile 1: >0 - <48.34 QPCR CCE/g)	436 (91.79)	39 (8.21)	1.14 (0.71-1.82)	1.00[d]
Yes (THETA tertile 2: ≥48.34 - <485.47 QPCR CCE/g)	323 (88.98)	40 (11.02)	1.75 (1.08-2.82)	1.57 (0.87-2.86)
Yes (THETA tertile 3: ≥485.47 - 21046.29 QPCR CCE/g)	275 (92.59)	22 (7.41)	1.29 (0.75-2.24)	1.16 (0.58-2.33)
Yes (THETA quartile 1: >0 - <25.88 QPCR CCE/g)	355 (90.33)	38 (9.67)	1.39 (0.87-2.22)	1.00[d]
Yes (THETA quartile 2: ≥25.88 - <315.12 QPCR CCE/g)	169 (88.02)	23 (11.98)	1.42 (0.74-2.72)	1.05 (0.50-2.20)
Yes (THETA quartile 3: ≥315.12 - <808.26 QPCR CCE/g)	287 (92.88)	22 (7.12)	1.27 (0.71-2.27)	0.92 (0.47-1.80)
Yes (THETA quartile 4: ≥808.26 - 21046.29 QPCR CCE/g)	223 (92.53)	18 (7.47)	1.40 (0.77-2.53)	1.03 (0.48-2.20)
	GI Illness			
Body Buried Status (Classification Category)	**No No. (%)**	**Yes No. (%)**	**aOR (95% CI)[a]**	**aOR (95% CI)[a]**
No	3932 (93.89)	256 (6.11)	1.00[b]	N/A
Yes (*B. thetaiotaomicron* (THETA) absent)	49 (92.45)	4 (7.55)	0.96 (0.33-2.77)	1.00[c]
Yes (*B. thetaiotaomicron* (THETA) present)	116 (88.55)	15 (11.45)	1.90 (0.91-3.96)	2.20 (0.56-8.67)
Yes (THETA ≤ median of 315.12 QPCR CCE/g)	92 (88.46)	12 (11.54)	1.71 (0.79-3.69)	1.00[d]
Yes (THETA > median of 315.12 QPCR CCE/g)	73 (91.25)	7 (8.75)	1.41 (0.48-4.11)	0.72 (0.20-2.55)
Yes (THETA tertile 1: >0 - <48.34 QPCR CCE/g)	62 (92.54)	5 (7.46)	0.97 (0.87-2.52)	1.00[d]
Yes (THETA tertile 2: ≥48.34 - <485.47 QPCR CCE/g)	54 (88.52)	7 (11.48)	1.98 (0.71-5.52)	2.24 (0.51-9.72)
Yes (THETA tertile 3: ≥485.47 - 21046.29 QPCR CCE/g)	49 (87.50)	7 (12.50)	2.08 (0.69-6.30)	2.07 (0.46-9.31)
Yes (THETA quartile 1: >0 - <25.88 QPCR CCE/g)	55 (91.67)	5 (8.33)	1.11 (0.42-2.90)	1.00[d]
Yes (THETA quartile 2: ≥25.88 - <315.12 QPCR CCE/g)	18 (78.26)	5 (21.74)	2.74 (0.77-9.69)	4.98 (0.84-29.42)
Yes (THETA quartile 3: ≥315.12 - <808.26 QPCR CCE/g)	50 (96.15)	2 (3.85)	0.64 (0.09-4.81)	0.51 (0.08-3.24)
Yes (THETA quartile 4: ≥808.26 - 21046.29 QPCR CCE/g)	42 (85.71)	7 (14.29)	2.70 (0.94-7.75)	1.85 (0.40-8.63)

[a]Robust variance estimates clustering on household. [b]Those who did not report the sand contact activity (digging; buried) are the reference category. [c]Those who reported the sand contact activity (digging; buried) with *B. thetaiotaomicron* absent are the reference category. [d]Those who reported the sand contact activity (digging; buried) with *B. thetaiotaomicron* present at the lowest concentration category are the reference category. aOR estimated from logistic regression model adjusted for age, sex, race/ethnicity, beach, and anycontact swimming.

TABLE 9. Relationship Between Continuous Fecal Indicator Measures and Risk of GI Illness by Sand Exposure Type.

	GI Illness			
	Restricted to those with sand contact		Uniformly low exposure assigned to those without sand contact	
	Digging	Body Buried	Digging	Body Buried
Fecal Indicator	**aOR (95% CI)[a]**	**aOR (95% CI)[a]**	**aOR (95% CI)[a]**	**aOR (95% CI)[a]**
Enterococcus (CFU/g)				
Per 1 $\log_{10}$ -unit increase	1.33 (0.86-2.05)	1.65 (0.43-6.34)	1.16 (1.04-1.28)	1.14 (0.92-1.41)
Enterococcus (QPCR CCE/g)				
Per 1 $\log_{10}$ -unit increase	1.45 (1.05-2.01)	3.12 (1.08-9.05)	1.11 (1.03-1.18)	1.13 (0.98-1.30)
Bacteroides (QPCR CCE/g)				
Per 1 $\log_{10}$ -unit increase	1.14 (0.87-1.48)	1.53 (0.89-2.63)	1.07 (1.00-1.15)	1.11 (0.98-1.26)
B. thetaiotaomicron (QPCR CCE/g)				
Per 1 $\log_{10}$ -unit increase	0.95 (0.79-1.14)	1.11 (0.73-1.68)	1.06 (0.99-1.13)	1.11 (0.97-1.26)

[a]Robust variance estimates clustering on household. aOR estimated from logistic regression model adjusted for age, sex, race/ethnicity, beach, and anycontact swimming.

TABLE 10. Relationship Between a Presence-Absence Index of All Five Fecal Indicators in Sand and GI Illness by Status of Sand Contact.

	GI Illness			
Digging Status (Categorical Classification)	**No No. (%)**	**Yes No. (%)**	**aOR (95% CI)[a]**	**aOR (95% CI)[a]**
No	3247 (94.69)	182 (5.31)	1.00[b]	N/A
Yes (Sum fecal index: 0-4 present)	299 (91.16)	29 (8.84)	1.19 (0.72-1.97)	1.00[c]
Yes (Sum fecal index: 5-9 present)	580 (91.77)	52 (8.23)	1.43 (0.93-2.21)	1.32 (0.69-2.52)
Yes (Sum fecal index: 10-15 present)	290 (90.34)	31 (9.66)	1.69 (1.03-2.78)	1.50 (0.76-2.98)
P for trend			0.02	0.245
	GI Illness			
Buried Status (Categorical Classification)	**No No. (%)**	**Yes No. (%)**	**aOR (95% CI)[a]**	**aOR (95% CI)[a]**
No	4229 (93.92)	274 (6.08)	1.00[b]	N/A
Yes (Sum fecal index: 0-4 present)	48 (94.12)	3 (5.88)	0.60 (0.20-1.85)	1.00[c]
Yes (Sum fecal index: 5-9 present)	100 (92.59)	8 (7.41)	1.41 (0.61-3.24)	2.83 (0.64-12.54)
Yes (Sum fecal index: 10-15 present)	42 (82.35)	9 (17.65)	2.84 (1.01-7.97)	5.48 (1.09-27.56)
P for trend			0.02	0.04

[a]Robust variance estimates clustering on household. [b]Those not in contact with sand (digging; buried) are the reference category. [c]Those in contact with sand (digging; buried) at the lowest fecal index category are the reference category. aOR estimated from logistic regression model adjusted for age, sex, race/ethnicity, beach, and anycontact swimming.

VI. CONCLUSIONS

A. Recapitulation of overall study aims and findings

The aim of the first phase of this study was to evaluate associations between two types of sand exposure with enteric (gastrointestinal GI illness and diarrhea) and nonenteric illnesses (upper respiratory illness [URI], skin rash, eye ailments, earache, and infected cuts) among participants at 7 beaches (4 freshwater and 3 marine). Comparison of the risk (incidence proportion) among beachgoers in contact with beach sand to the risk among beachgoers not in contact with beach sand was made (including among a sub-group of children ≤10 years). The risk of GI illness among those digging in the sand was 1.14 times the risk of GI illness among those who did not dig in the sand (95% CI = 1.02-1.26). A stronger association was observed among those buried in the sand, which was observed to be a more intense sand contact exposure. The risk of GI illness among those buried in the sand was 1.22 times the risk of GI illness among those not buried in the sand (95% CI = 1.05-1.36). A slight elevation in the risk of GI illness was observed in a sub-group of children ≤10 years who dug in the sand (aIPR = 1.21; 95% CI = 0.97-1.51) and who were buried in the sand (aIPR = 1.21; 95% CI = 0.94-1.63). We observed a stronger positive association between sand contact activities and a more narrow/specific definition of enteric illness (diarrhea). The adjusted risk of diarrhea among those digging in the sand was 1.20 times the adjusted risk of diarrhea among those not digging in the sand (95% CI = 1.05-1.36). This association with diarrhea was elevated for those buried in the sand (aIPR = 1.23; 95% CI = 1.01-1.51) and among children ≤10 years who dug in the sand (aIPR = 1.45; 95% CI = 1.01-

2.09) and buried in the sand (aIPR = 1.23; 95% CI = 0.94-1.63). Associations with nonenteric illnesses were not strong or consistent for both the sand exposures. The results of the first phase of this research are supported by a recent finding that time spent in contact with wet sand was associated with an increased risk of GI illness.[41]

Given the consistently positive associations observed between sand contact activities and enteric illnesses, it was hypothesized that fecal indicators of microbial sand quality may be associated with enteric illness (specifically GI illness). The aim of the second phase of research was to evaluate associations between 5 fecal indicator measures of beach sand quality (*Enterococcus* [CFU/g], F^+-specific coliphage [PFU/g] *Enterococcus* [qPCR CCE/g], *Bacteroides* [qPCR CCE/g], *B. thetaiotaomicron* [qPCR CCE/g]) collected at 3 transects at 2 marine beaches, 2 types of sand contact (digging in sand; buried in sand), and GI illness. A consistently positive association was observed between a novel molecular *Enterococcus* measure and GI illness for both sand quality exposures.. The risk (odds) of GI illness among those digging in sand in the highest tertile of *Enterococcus* exposure (>48.87 qPCR CCE/g) was 1.95 times the risk (odds) of GI illness among those not digging in the sand (95% CI = 1.23-3.09). This association with GI illness was stronger among those who buried their body in the sand (aOR = 3.49; 95% CI = 1.43-8.50). This association with GI illness was also robust to various categorical classifications of the *Enterococcus* qPCR CCE measure suggesting validity of an assumption of linearity. Overall, there was no strong or consistent association with GI illness for the other fecal indicator measures using categorical classifications.

Results of continuous classification showed that among participants who reported digging in the sand, a 1 $\log_{10}$ increase in the daily *Enterococcus* qPCR CCE average resulted

in a 1.45 increase in the risk (odds) of GI illness (95% CI = 1.05-2.01). The relationship was stronger among participants buried in the sand (aOR = 3.12; 95% CI = 1.08-9.05). This association was robust to re-assignment of a uniformly low value to those not in contact with sand (i.e., not digging; not buried) which resulted in a substantial improvement in precision (but a decrease in the magnitude of the association).

Overall the results of the second phase of research suggest a consistently positive association between *Enterococcus* qPCR CCE levels in sand and GI illness among participants engaged in sand contact activities. Use of an index of the presence/absence of all 5 fecal indicators summed across the 3 transects also showed a consistent positive association with GI illness.

B. Strengths

This research combined a first phase that permitted the evaluation of associations between both types of reported sand contact activities with a number of health endpoints among a large cohort of beach-goers (n=26,339 individuals). The first phase elucidated consistently positive associations between both sand contact activities and enteric illness (GI illness and diarrhea). These findings informed the second phase of research which was to determine whether continuous measures of microbial sand quality were associated with enteric illness (specifically GI illness) for the same two sand contact activities. The traditional and novel fecal indicator measures studied included one considered to be able to re-grow in sand (*Enterococcus*) and 3 others considered to be more human-specific indicators of fecal contamination (F^+-specific coliphage, *Bacteroides*, and *B. thetaiotaomicron*). The quantification methods included traditional culture-based methods (EPA Method 1600, EPA Method 1601) and a novel, rapid (2-4 hours) quantitative molecular method (qPCR CCE).

The positive association observed between *Enterococcus* qPCR CCE and GI illness was consistent across sand exposures and estimation approaches. To the best of our knowledge, this is the first study to demonstrate an association between sand contact activities and GI illness as a function of microbial sand quality.

C. Limitations

Although the first phase of research represents the largest and most comprehensive investigation of associations between sand contact and risk of illness (26,339 individuals at 4 freshwater beaches and 3 marine beaches), the analysis of the less frequent sand exposure (buried in sand), less frequent outcomes (non-enteric illnesses), and the analysis among a sub-group of children ≤ 10 years old (and other age sub-groups) was limited by small sample sizes. The second phase of research (of 4,838 individuals at 2 marine beaches) was also limited by small sample sizes in sand exposure groups (especially among those buried in sand). This limited our ability to examine variation in effect estimates by sand exposure, sub-groups of age (and other covariates such as beach and swimming status) and also limited the number of covariates that could be considered as confounders in multivariate regression models. The smaller sample size of the second phase of research led to instability of log-linear binomial regression models and limited our ability to estimate the incidence proportion as a measure of risk. We therefore used logistic regression models to estimate odds ratios, adjusted for critical covariates. The health endpoints examined during both phases of this research were broad and in some cases non-specific and could have been affected by recall bias. However, we expected that recall of illness would have been nondifferential with respect to sand exposure status given that the NEEAR Water Study was focused on illness related to swimming exposure. Nondifferential recall of this kind likely resulted in unbiased

estimates, although there could have been a loss of precision if there was under-recall (or reporting) of illness among participants exposed to sand.

D. Future directions

It is not clear whether the relationships observed at these beaches can be extended to beaches affected primarily by non-point sources of fecal contamination. Future studies should be conducted at beaches influenced by diffuse sources of fecal contamination (i.e., urban run-off, wild birds, other animal populations, human bathers). Evaluating relationships at beaches impacted by diffuse pollution sources and also at freshwater beaches may help clarify the extent to which specific sand quality indicators are associated with GI illness and nonenteric illnesses among those with sand contact.

Microbial source tracking methods may help discern the predominant sources of fecal contamination in beach sand and lead to a better understanding of relationships between microbial sand exposure and health effects. One novel fecal indicator measure, *Clostridium perfringens*, is considered to be a more specific indicator of fecal contamination in sand, and its inclusion in future recreational water studies may be advisable. A goal of future studies should be to improve the ability to determine/type specific sources of fecal contamination in beach sand. This could lead to more refined exposure classification of participants in contact with sand on days when human-specific microbial pollution is detected. Several novel methods of fecal indicator measurement show promise, including a culture, latex agglutination, and typing assay as well as reverse transcriptase and reverse line blot hybridization assays (which have been applied to F^+-specific coliphage in recreational water samples). These methods could be used to serogroup F^+-specific coliphage isolated from beach sand into human vs. non-human types. The incorporation of typing information with

quantitative fecal indicator measures could lead to a more refined sand exposure classification than was possible in the present study.

It is unknown whether the relationships we observed between *Enterococcus* qPCR CCE and GI illness can be extended to more specific definitions of GI illness (diarrhea, vomiting) or nonenteric illnesses (upper respiratory illness, eye irritation, skin rash, earache, and infected cuts/wounds). It appears advisable to continue to investigate the relationship between microbial sand quality and a more diverse number of health endpoints among those in contact with sand. Given the broad nature of some of the health endpoints considered during this research, improvements in outcome classification could be made by incorporating a salivary antibody test (multiplex Luminex^{TM} immunoassay; Enzyme-Linked ImmunoSorbent Assay [ELISA]) of seroprevalence for specific viral, bacterial, and parasitic infections considered to cause the majority of enteric illness (including Norovirus, Rotavirus, *Giardia lamblia*, *Cryptosporidium*, and *Helicobacter pylori*). Collection of saliva samples offers a non-invasive method that may be practical to incorporate into future recreational water and sand quality studies.

Replicating our investigation of microbial sand quality at beaches influenced by non-point sources and at freshwater beaches as well as improving exposure and outcome measurement and classification in future studies could clarify relationships between microbial sand quality and illness among those in contact with sand. These improvements could help elucidate whether development of sand quality guidelines protective of public health is necessary.

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